## *The effects of climatic warming on the phenology of Arctic plants and their principal herbivore*

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by

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# The effects of climatic warming on the phenology of Arctic plants and their principal herbivore

***Epirrita autumnata* larva on *Betula pubescens* ssp. *tortuosa***

## Abstract

### The effects of climatic warming on the phenology of Arctic plants and their principal herbivore

**M.Sc. Thesis by Claire L. Flockhart**

There is mounting evidence that many plants and animals may be unable to adjust the timing of their various life cycle events rapidly enough to adjust to predicted rates of climate change. For example, the close synchronisation between bud-burst and herbivore emergence may be disrupted by increased temperatures. However, many experimental studies demonstrating these effects have only used short term temperature manipulations, despite the possibility that strong selection pressure could lead to rapid adaptation to a changing climate over several years. This study therefore investigated whether phenological synchrony between plants and their herbivores was disrupted after five years of simulated climate change.

A series of warming chambers was set up across the forest-tundra ecotone in Arctic Sweden. The chambers increased temperatures relative to control plots in line with average predictions from climate change models. The warming chambers did not affect the timing of snow melt, suggesting that hydrological conditions were broadly similar in warming chambers and control plots.

Plants growing in areas with a late-melting snow pack showed accelerated development and growth in comparison with plants in early-melting areas, so that plants in all areas completed development at about the same time. However, invertebrate herbivores were more closely synchronised to their host plants in areas where immature foliage was only available for a short period of time. Experimental warming advanced the onset of leaf development and also increased the area of leaf tissue available to herbivores. However, phenological synchrony between invertebrate herbivores and their host plants was not affected by warming, as the lag between leaf emergence and the onset of herbivory was similar in warming chambers and control plots. This suggests that herbivores may be able to respond relatively rapidly to changes in host plant phenology. However, elevated temperatures were associated with an increase in defoliation, with potentially severe consequences for the birch forests of northern Fennoscandia.

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For my friends and family, particularly my parents and Rich, for all their support and encouragement

# Chapter 1 – General Introduction

The possible effects of human induced climate change on ecosystems have received a large amount of research effort in recent years. Furthermore, public and political concern about climate change has reached a high level. Current concerns have been fuelled by the predictions made from global circulation models (GCMs), which suggest that global mean temperatures will increase throughout the 21st century (Houghton et al., 1996). Although changes in global climate are not a new occurrence, the rate at which these changes are predicted to occur is greatly increased as a result of anthropogenic activities (Huntley, 1991; Maxwell, 1992). Therefore, even if species were previously capable of tracking or adapting to changes in the climate (Parmesan, 1996; Parmesan et al., 1999), the current rate of change may commit species to extinction even if they are able to adapt rapidly to the new climatic conditions (Thomas et al., 2004). Determining the rate of adaptation to changes in climate for species at different trophic levels has therefore become a pressing issue in order to understand exactly how the predicted changes in climate will affect modern biodiversity.

This thesis reports the findings of a study undertaken to determine the effects of climatic warming on the phenology of Arctic plants and their principal herbivores. Because the survival of invertebrate herbivores is largely dependent on the availability of palatable food, it is vital that the timing of their life cycles is closely synchronised with that of their food plants (Bale et al., 2002). However, because warmer climatic conditions are expected to advance the timing of phenological events, the current synchronisation between invertebrate herbivores and their host plants may be disrupted (Hughes, 2000). The present study therefore investigated whether invertebrate herbivores are able to remain closely synchronised to their host plants when significant climatic warming is simulated. The effects of different altitudes and habitat structures on the timing of life cycle events were also examined.

Many interactions across different trophic levels rely on life-history events being closely synchronised. For example, because lepidopterous larvae feed preferentially on young deciduous leaves, the timing of egg hatch is closely matched to that of bud-burst, allowing the larvae to fully exploit the resource. If bud-burst and larval eclosion were not synchronised, larvae that hatch too early may starve (Wint, 1983; Hunter, 1990; Watt & McFarlane, 1991), while those that hatch after bud-burst will be forced to consume leaf tissues that have a lower nutritious value and that are less digestible. In addition, late-hatching individuals will be subject to intense competition from conspecifics in more advanced stages of development (Feeny, 1970; Ayres & MacLean, 1987; Watt, 1987; Kaitaniemi et al., 1997). In either of these scenarios, where the length of the larval period is increased, there is also an elevated risk of predation and parasitism (Virtanen & Neuvonen, 1999).

Determining the processes by which Arctic invertebrate herbivores are able to synchronise their development with that of their hosts has been the subject of intense investigation as a result of the devastating defoliating outbreaks common in the birch forests of Fennoscandia (Sonesson & Callaghan, 1991; Virtanen et al., 1998; Klemola et al., 2003). Outbreaks of *Epirrita autumnata* (Borkhausen) occur approximately every nine to ten years within the Fennoscandia mountain chain and generally have no long-term effects on the ecosystems (Tenow, 1996). At times, however, trees have been killed over vast areas thereby affecting the position of the forest line (Tenow & Bylund, 2000). Therefore, if the current synchrony between invertebrate herbivores and their host plants is disrupted by climate change, there may be severe consequences for the position of the forest-tundra ecotone.

Existing studies have suggested that one of the main manifestations of climate change will be an increase in mean temperatures (Bale et al., 2002). Although this will ameliorate and extend the growing season for many plants (Ayres, 1993; Chapin et al., 1995; Press et al., 1998), it may also disrupt the synchrony between invertebrate herbivores and their food plants (Harrington et al., 1999; Hughes, 2000; Whittaker, 2001). Dewar and Watt (1992), for example, concluded that an increase in temperature of 2 – 5 °C would lead to

an increase in phenological asynchrony between *Picea sitchensis* (Bongard) Carrière[1] and the winter moth, *Operophtera brumata* (Linnaeus). The evidence is, however, somewhat contradictory as Buse and Good (1996) demonstrated that phenological synchrony between *Quercus robur* L. and *O. brumata* would not be disrupted by a 3 °C increase in temperature. Although recent reviews have suggested that these contrasting conclusions may be the result of different methodologies, such patterns may also be the result of differing emergence mechanisms in different geographic locations (Harrington et al., 1999; Watt & McFarlane, 2002; Dixon, 2003).

As a result of the complex nature of insect and plant life cycles, the spring and winter temperature profiles experienced by insects and their host plants are generally different (Watt & McFarlane, 2002). Despite the different cues available, invertebrate herbivores are generally able to track the life-cycles of their hosts and thereby remain in synchrony. With climate change, however, these intertrophic relationships may be disrupted since the requirements to commence growth often differ qualitatively between species (Watt & McFarlane, 2002). For example, Dewar and Watt (1992) showed that insects without a winter chilling requirement can synchronise their emergence with bud-burst in a host plant which requires winter chilling. However, climate change may mean that winter temperatures may not be sufficiently low in the future to meet winter chilling requirements, thereby disrupting host plant phenology. As eggs which hatch at the time of bud-burst will have a higher fitness than those that hatch earlier or later, one may expect any disruption in synchrony to be corrected for by selection (Komatsu & Akimoto, 1995), but how quickly selection can act to ensure continued synchrony is unknown.

One of the research priorities identified by Harrington et al. (1999) is to determine the relative speed that different trophic levels can adapt to a changing climate. However, relatively few studies involve long term manipulations of natural systems, so the present study takes advantage of a

[1] Nomenclature of all higher plants follows Tutin, T. G., Heywood, V. H., Burges, N. A., Valentine, D. H., Walters, S. M. & Webb, D. A. 2001. Flora Europaea. Cambridge: Cambridge University Press.

chamber-based warming experiment that has been in place for five years. Although the increased temperatures in experimental plots may initially have had a strong effect on the phenological synchrony between herbivores and their host plants, the present study aimed to determine whether the insect populations were able to adapt to the new conditions after selection had acted over several generations. As the effects of climate change are predicted to be greatest at higher latitudes, understanding how this will affect trophic interactions has become vital (Keeling et al., 1996; Press et al., 1998). Furthermore, since the conditions in Arctic regions are already close to the limits for sustaining life, even relatively small changes should produce highly visible effects (Danks, 1992)

## 1.1 Thesis outline

Before determining whether phenological synchrony between invertebrate herbivores and their host plants can adapt to long term climatic warming, it is vital to establish how plant phenology responds to natural variation in the length of the growing season. Chapter 3 therefore relates the timing of plant life-history events to the natural environmental conditions. Although the timing of snow melt differed across the forest-tundra ecotone, plants located in late melting areas increased the rate of their development relative to those in early melting areas. Given that plants were able to adjust the rate of their development to the length of the growing season, did this have implications for their associated herbivores? Chapter 4 therefore investigates the natural patterns of invertebrate herbivory and synchrony across different habitats and altitudes that were known to vary in growing season length. Invertebrate herbivory across the forest-tundra ecotone appeared to be largely controlled by the availability of palatable leaf tissues with closer synchrony in the late-melting forest habitats.

An increase in temperatures is believed to be one of the main consequences of climate change. Chapter 5 therefore investigates whether the natural pattern of phenology in Arctic plants and their herbivores is disrupted by

warming. The results demonstrated that experimental warming advanced the onset of leaf development as well as increasing the area of leaf tissue available to herbivores. However, phenological synchrony between invertebrate herbivores and their host plants did not appear to be disrupted.

# Chapter 2 – General Methods

## 2.1 General site information

The study sites were located within the Abisko National Park (68°19`N, 18°51`E) in northern Sweden (figure 2.1). The landscape was dominated by the tree *Betula pubescens* ssp. *tortuosa* (Ledeb.) Nyman grading to tundra at approximately 570 m a.s.l. The forest-tundra ecotone was characterised by continuous *B. pubescens* ssp. *tortuosa* forest giving way to small patches of forest and finally becoming open tundra. Forest habitat comprised more or less continuous canopy cover at about 2.5 m height and it lacked a shrub layer. The prostrate ground flora was dominated by *Vaccinium* species including the deciduous *V. myrtillus* L. and *V. uliginosum* L. and the less frequent evergreen *V. vitis-idaea* L. together with the bryophyte *Hylocomium splendens* (Hedwig). There were also many rarer species, principally graminoids, *Carex* spp. and *Salix* spp. The tundra habitat was characterised by an entirely prostrate flora, not exceeding 30 cm in height, dominated by *Empetrum nigrum* L., *V. vitis-idaea*, *Betula nana* L. and bryophytes.

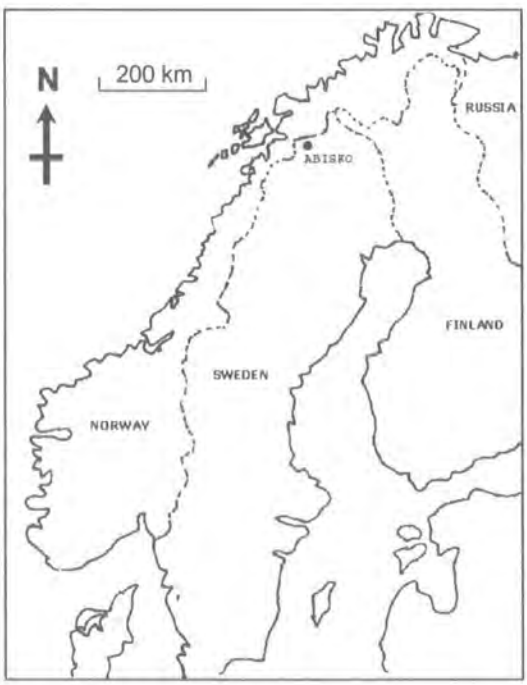


**Figure 2.1: Map showing location of study area – Abisko National Park, Sweden.**

Four sites were set up across the forest-tundra ecotone in June 1998. Sites were established in continuous forest at 520 m a.s.l. and in continuous tundra at 600 m a.s.l. A further two sites were established in a tundra patch within mainly forest habitat at 550 m a.s.l., and in a forest patch within mainly tundra habitat at 600 m a.s.l. A schematic of the study area is shown in figure 2.2 together with site names and an example of the layout of plots within each site. A further site (lake-forest site) was established in *B. pubescens* ssp. *tortuosa* forest at 360 m a.s.l. to investigate patterns of herbivory in that species.

Five open-top chambers (OTCs) and control plots, each with an area of 1 $m^2$, were placed in each of the four sites. The control plots were marked out with twine (figure 2.3) while the hexagonally shaped OTCs were made from 5 mm thick UV-stabilised clear polycarbonate sheet (figure 2.4). All plots were established on flat ground that had no standing water.

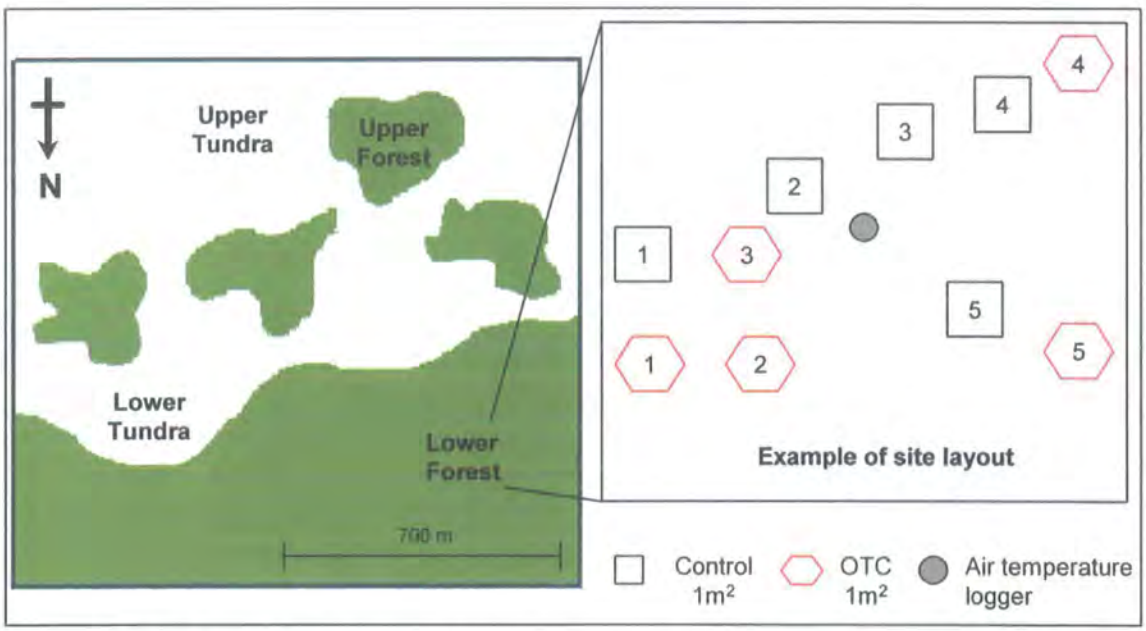


**Figure 2.2: Schematic of study area with an example of a site layout. The birch forest is shown in green and the tundra in white.**

**Figure 2.3: An example of a control plot (lower right hand side), with two OTCs in the distance, in the lower forest site. The control plots had an area of 1 $m^2$ and were marked out with twine. Photograph taken April 2003.**

**Figure 2.4: An example of an open-top chamber in the lower tundra site. The OTC had an area of 1 $m^2$ at the base and reached approximately 50 cm off the surface of the ground. The OTCs were secured with guy ropes to prevent movement. Photograph taken April 2003.**

The OTCs and control plots were matched on the basis of slope, aspect and species composition, thereby allowing paired statistical tests to be undertaken (Huntley, 2002).

## 2.2 Study species

### 2.2.1 *Betula nana* L.

*Betula nana* is a prostrate deciduous shrub that can reach up to 1 m in height (De Groot et al., 1997). It has a circumpolar distribution extending to the western coastal regions of Greenland, Iceland, the British Isles, northern Europe and western Siberia (Hulten & Fries, 1986). In Scandinavia, it occurs in the northern belt of the subarctic tundra between 20 – 1075 m (De Groot et al., 1997). The timing of bud-burst is strongly influenced by air temperatures once snowmelt has occurred (Hannien, 1990; Pop et al., 2000), and a period of winter chilling is required before bud-burst will occur in response to spring warming (Wareing, 1956; Perry, 1971). As the growing season length is rarely more than 100 days in Arctic regions (Miller, 1982), leaf development occurs very rapidly with autumn leaf senescence commencing in July as a result of a change in photoperiod length and a drop in temperature (Eriksson & Jonasson, 1986).

### 2.2.2 *Vaccinium myrtillus* L.

*Vaccinium myrtillus* is a deciduous rhizomatous shrub that can reach 10 – 60 cm in height (Ritchie, 1956). It has a wide ranging distribution extending from the British Isles through northern and central Europe to northern Asia (Hulten & Fries, 1986). Abundantly distributed as an understorey shrub in natural and semi-natural woodlands its altitudinal limit depends on the degree of protection offered by snow cover (Ritchie, 1956; Preston et al., 2002). Bud-burst is largely controlled by degree-days (Theurillat & Schlussel, 2000) and tends to occur simultaneously with birches (Klemola et al., 2003).

### 2.2.3 *Epirrita autumnata* (Borkhausen)

The geometrid moth *Epirrita autumnata,* is a polyphagous leaf-chewing herbivore whose larvae feed preferentially on young deciduous foliage in the spring (Klemola et al., 2003). The species' range extends throughout western Europe and most of northern Eurasia (Ayres & MacLean, 1987) and it is univoltine in northern Scandinavia. The larvae pupate in the soil after five larval instars (Haukioja et al., 1988). The flight period for the species lasts between three and five weeks depending on the length of the pupal period (Kaitaniemi et al., 1999; Tammaru et al., 2001). A female lays her eggs, mostly singly, within a few days on the branches and stems of the foodplants (Tammaru et al., 1995) where they remain dormant throughout the winter. The eggs of *E. autumnata* die at temperatures below -36°C in mid-winter and below -29°C during post-diapause embryogenesis (Tenow & Nilssen, 1990). The date of emergence depends on degree-day accumulation and is therefore strongly related to the date of oviposition the previous autumn (Ruohomaki et al., 1993; Kaitaniemi et al., 1999). Emerging larvae frequently disperse from their natal site by spinning silk threads as egg-laying females tend to oviposit indiscriminately in relation to availability of food for emerging larvae. *Epirrita autumnata* feeds primarily on *B. pubescens* ssp. *tortuosa* but is also known to consume both *B. nana* and *V. myrtillus* (Ayres & MacLean, 1987; Klemola et al., 2003).

## 2.3 Sampling procedure

One of the common prostrate deciduous plant species in each habitat was used to determine the impacts of climate change on the phenology of Arctic plants and their associated herbivores. These were *B. nana* in tundra habitats and *V. myrtillus* in forest habitats. At each site the relevant plant species was used to obtain measurements of phenology, leaf growth and patterns of invertebrate herbivory. The extent of invertebrate herbivory on an abundant evergreen, *V. vitis-idaea*, was also measured in both OTCs and control plots.

Measurements at the five sites across the forest-tundra ecotone were taken in a total of nine sampling sessions between 23 May and 1 July 2003. It took up to four days to collect the data from all sites during each sampling session, so for statistical analyses the median date of each sampling period was used (table 2.1).

**Table 2.1: Dates of data collection for the present study between 23 May and 1 July 2003. The upper tundra, upper forest, lower tundra and lower forest were visited once per session while the lake-forest site was visited twice per session.**

| Session number | Start of session | End of session | Median date |
|---|---|---|---|
| 1 | 23 May | 25 May | 24 May |
| 2 | 27 May | 29 May | 28 May |
| 3 | 2 June | 2 June | 2 June |
| 4 | 6 June | 8 June | 7 June |
| 5 | 10 June | 12 June | 11 June |
| 6 | 14 June | 16 June | 15 June |
| 7 | 19 June | 23 June | 21 June |
| 8 | 25 June | 27 June | 26 June |
| 9 | 29 June | 1 July | 30 June |

Due to prolonged snow cover in both the upper and lower forest sites, many of the plots were snow covered at the beginning of the measurement period. Measurements were only taken once plots were at least partially snow-free. For statistical analyses, the phenological stage in deciduous species and the extent of herbivory on *V. vitis-idaea* in the first snow-free period was assumed to be the same as those prior to the first sampling period; i.e. both leaf development and invertebrate herbivores were assumed to be inactive until the plots were snow-free.

## 2.4 Leaf area calculations

This study relies on measuring leaf area availability in relation to herbivory. To avoid biasing measurements of herbivory, it was necessary to minimise disturbance to leaves within plots as they were growing, as this could disrupt

natural patterns of herbivory. For example, a hand-held leaf area meter could not be used *in situ* as this would be likely to disturb herbivores on the leaves. It was therefore necessary to develop a method of predicting leaf area from measurements that could be obtained without causing undue disturbance to leaves or herbivores. One such measurement is leaf length, which could be easily obtained using digital callipers by simply offering up the calliper tips close to the leaf.

The relationship between leaf length and area was investigated by using digital callipers to measure the length of one hundred fully unfurled and undamaged leaves of *B. nana, V. myrtillus* and *B. pubescens* ssp. *tortuosa.* Leaves for this analysis were taken from plants just outside the experimental plots. After measurement, each leaf was collected and placed in a waxed paper envelope marked with a unique reference number. Later the same day, each leaf was digitised using a scanner at a resolution of 600 dpi, and analysed with the SigmaScan Pro software (SPSS, Chicago, Illinois, USA). The software was used to obtain precise measurements of the area of each leaf. A regression model was fitted to the relationship between the original length measurements and the calculated leaf area, thereby allowing the area of leaves within experimental plots to be estimated when only their length was known (equations 1, 2 & 3; figure 2.5).

*Betula nana*

leaf area = 9.88 × leaf length – 32.27

$R^2 = 0.83$, d.f. = 98, $p < 0.001$ **Equation 1**

*Vaccinium myrtillus*

leaf area = 8.29 × leaf length – 29.18

$R^2 = 0.88$, d.f. = 98, $p < 0.001$ **Equation 2**

*Betula pubescens* ssp. *tortuosa*

leaf area = 25.25 × leaf length – 225.71

$R^2 = 0.83$, d.f. = 97, $p < 0.001$ **Equation 3**

Where leaf area was measured in $mm^2$ and leaf length in mm.

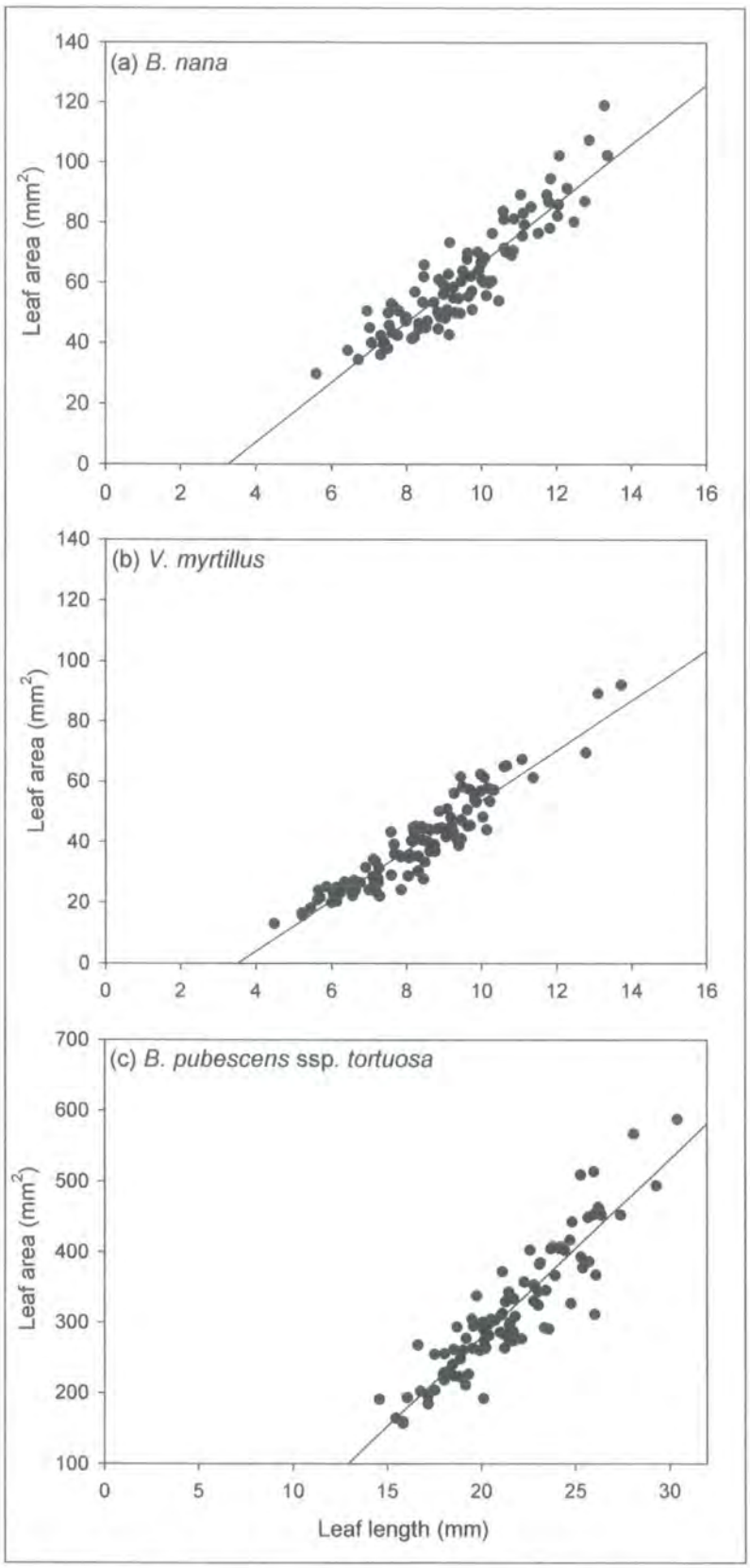


**Figure 2.5: Relationship between leaf length and leaf area for (a) *B. nana*, (b) *V. myrtillus* and (c) *B. pubescens* ssp. *tortuosa*. Equations for regression lines are shown on page 12.**

The models had negative intercepts, suggesting that leaf growth was non-linear in the early stages. However, figure 2.5 shows that leaf growth was very close to linear through the range of leaf lengths sampled. This indicates that the regression model was sufficiently powerful to predict accurately leaf area from leaf length as measured by digital callipers in the field (all $r^2 > 0.8$).

# Chapter 3 – Temperature regimes and plant phenology

## 3.1 Introduction

The timing and rate of phenological development in Arctic plants are controlled primarily by the physical environment, in particular temperature regimes and the length of the growing season (Bliss, 1956; Chapin & Shaver, 1985; Billings, 1987; Hannien, 1990; Kudo, 1991; Shaver & Kummerow, 1992; Aizen & Patterson, 1995; Stanton et al., 1997). However, the physical environment in Arctic regions is highly unpredictable both within and among years. For example, the interaction between wind and topography causes irregular snow distributions throughout the winter months, leading to substantial local differentials in winter temperature profiles and melt patterns, which can affect plant phenology (Billings & Bliss, 1959; Billings, 1987; Sonesson & Callaghan, 1991). One major source of variation is the physical structure of Arctic habitats. Habitats under a canopy cover may retain extensive areas of snow that does not melt until relatively late in spring, whereas snow cover on exposed habitats may be thin or absent as a result of redistribution by wind action (Danks, 1991). Prolonged snow cover has been shown to restrict subsequent plant growth (Billings & Bliss, 1959), but there is little published information on the effect of variation in snow cover, mediated by habitat structure, on the phenology of Arctic plants. This will have important implications for patterns of food availability for Arctic herbivores, and may affect the position and dynamics of the forest-tundra ecotone (Pop et al., 2000).

The factors that initiate plant growth activity in the spring are still unclear, although increasing air temperature is believed to be one of the most important (Van Wijk et al., 2003). For example, plant growth in north-east Greenland did not begin until almost the same time as mean daily soil surface temperatures rose above 0 °C (Sorensen, 1941), and numerous studies have shown that snow cover affects the timing of bud-burst (Bliss, 1956; Kudo, 1991; Sonesson & Callaghan, 1991; Galen & Stanton, 1993;

Price & Waser, 1998; Van Wijk et al., 2003). However, as the pattern of snow melt varies dramatically across the forest-tundra ecotone, it is clear that the date at which plant growth will start in relation to air and soil temperatures will also be constrained in various ways in different components of the landscape mosaic.

The growing season for Arctic plants is much shorter than that for temperate and tropical plants (Sakai & Larcher, 1987). Furthermore, within Arctic systems, one might expect plants in areas of prolonged snow cover to be at a disadvantage in the time available for resource assimilation and growth in comparison with those in areas of limited snow cover. It has been suggested that plants may compensate for such a reduction in growing season length by facultatively increasing the rate of their development (Sorensen, 1941; Galen & Stanton, 1995). Such compensatory growth may be enhanced by the fact that immediately after snow melt, plants have sudden access to full light intensity, relatively high air and soil temperatures, and abundant soil moisture allowing late starting leaves to grow rapidly. *Salix herbacea* L. leaves, for example, can attain 75% of their total leaf area within two weeks of snow melt (Wijk, 1986). Furthermore, plants released from snow cover very early in the season may suffer drought (because the soil may still be frozen) and frost damage (Cannell & Smith, 1986; Starr et al., 2000).

The evidence for plants compensating for a late start in this way is somewhat ambiguous. Wijk (1986) showed that the phenological development of *S. herbacea* occurred at the same rate irrespective of snow release dates, so that late-starting plants were always behind early-starting plants. Similarly, the rate of leaf expansion of *Quercus ilicifolia* Wangenh. did not change along a temperature gradient in eastern North America (Aizen & Patterson, 1995). In contrast, several alpine plant species (*Geum rossii* (R. Brown), *Trifolium parryi* A. Gray, *Poa alpine* L., *Ranunculus adoneus* A. Gray, *Sibbaldia procumbens* L.) located in late-melting areas showed increased rates of leaf expansion in comparison with conspecifics in early melting areas (Galen & Stanton, 1995). In other words, plants in regions of prolonged snow cover compensated for the reduction in growing season length by

growing at a faster rate than those in early melting regions. Galen & Stanton (1995) also confirmed earlier findings that time to flowering was reduced in plants released from snow cover at later dates (Billings & Bliss, 1959).

### 3.1.1 Hypotheses

#### 3.1.1.1 The effect of snow cover on soil surface temperature profiles

Soil surface temperature profiles have been shown to be very important for plant and herbivore development (Cannell & Smith, 1986; Billings, 1987; Bale, 2002; Van Wijk et al., 2003). One important factor driving soil surface temperature patterns is the thickness and duration of the snow pack (Billings & Bliss, 1959). The present study tested the hypothesis that tundra habitats will show greater fluctuations in early spring soil surface temperatures than forest habitats, and will provide plants with an extended growing season in comparison with forest habitats. On open tundra habitats, where snow cover is relatively thin, the soil surface is predicted to gain little protection from sub-zero air temperatures. However, in forested habitats, where the sheltered conditions might lead to the formation of a thick isothermic snow pack, the soil surface would be protected from large variations in air temperature. Furthermore, a thick snow pack is also predicted to have the consequence of reducing growing season length, owing to the time required to melt the snow pack in spring. These effects could lead to important differences between the two habitats for plant and invertebrate development.

#### 3.1.1.2 The effect of soil surface temperature profiles on plant phenology

The onset of plant development is related closely to ambient temperatures rising in spring (Sorensen, 1941). Leaf emergence has been shown to commence early in areas covered by a thin layer of snow which subsequently dissipates rapidly once temperatures rise above freezing (Billings & Bliss, 1959). However, in areas covered by a dense isothermic

snow pack, bud-burst is frequently delayed as a direct result of the length of time required to melt the snow.

Plant responses to physical conditions have important consequences for the life history strategies of their associated herbivores. Therefore, this study tested the hypothesis that plants released early from snow cover will commence their development earlier in the season. Patterns of leaf development of *Betula nana* and *Vaccinium myrtillus* were recorded across the forest-tundra ecotone. In open tundra habitats, where snow cover is known to be limited, we would predict leaf emergence to commence earlier than in forest habitats, where the date of release from snow cover is extended by the time taken to melt a dense isothermic snow pack.

#### 3.1.1.3 The effect of growing season length on leaf growth rates

Although Arctic plants are well adapted to a relatively short growing season (Sakai & Larcher, 1987), 'late-starting' plants (i.e. those in areas of late snow melt) will be at a disadvantage in comparison with 'early-starting' plants. However, it is possible that later starting plants could compensate for their shorter growing season by growing more rapidly when they are finally released from snow cover. If such compensatory growth occurs, folivores may not be disadvantaged in late starting areas relative to early starting areas. However, an accelerated growth rate may reduce the nutritional content of the leaves.

Therefore, this study tested the hypothesis that plants subject to a shorter growing season will show increased rates of leaf development to compensate for their late start. Leaf growth rates were monitored in forest and tundra habitats. The compensatory growth hypothesis predicts that leaf growth should occur more rapidly in forest habitats (late snow dissipation) than in tundra habitats (early snow dissipation).

In summary, because forested habitats are predicted to have a thick isothermic snow pack, offering a high level of protection from ambient

temperatures, they are also predicted to have a shorter growing season when compared to tundra habitats. Consequently, plant growth in these late-melting forested habitats is predicted to be delayed in relation to early melting tundra habitats. Finally, as a longer period of time is available for growth in early melting tundra habitats the rate of leaf growth is predicted to be slower than in areas where the growing season length is reduced by late-lying snow cover.

## 3.2 Methods

### 3.2.1 Weather measurements

Shielded air temperatures at 2 m above the ground surface were recorded at hourly intervals at the four sites, two in tundra habitats and two in forest habitats (Tinytag Plus Gemini Data Loggers, Chichester, West Sussex, UK; see section 2.1 for details of the sites). As previous snow packs were less than 2 m in height these loggers were expected to remain well above the snow layer, thereby recording actual air temperature throughout the year. An unshielded logger was placed at ground level in a randomly selected control plot at each of the sites, to record temperatures experienced by understorey/dwarf shrub plants and invertebrate species. Loggers were positioned before snow fall in autumn 2002 allowing both air and soil surface temperatures to be recorded between 1 January 2003 and 14 June 2003. Unfortunately, data were not recorded beyond 14 June 2003 due to memory limitations of the loggers. Hourly air and soil surface temperatures were pooled to calculate daily mean, maximum and minimum temperatures, with associated standard deviations.

Hours of sunshine, wind speed and precipitation records were obtained from a weather station located approximately 4 km away from the study sites at the Abisko Scientific Research Station (68°21`N 18°49`E, Abisko Naturvetenskapliga Station, Abisko SE – 98107, Sweden). Hours of sunshine and wind speed were recorded between 1 January 2003 and

14 June 2003. Precipitation was collected in a heated rain gauge, but due to a technical problem, beyond the control of the author, was only recorded between 1 January 2003 and 20 March 2003. Daily mean, maximum, minimum and associated standard deviations were calculated for sunshine, wind speed and precipitation.

### 3.2.2 Plant phenology

The phenological processes of bud-burst and leaf growth were recorded in all sites across the ecotone. *Betula nana* was measured on the upper and lower tundra sites and *V. myrtillus* was measured on the upper and lower forest sites. Each plot was visited at approximately four day intervals between the end of May 2003 and the beginning of July 2003 (see table 2.1, page 11 for dates of each sampling session) resulting in nine sampling sessions during the growing season. If a plot was fully snow-covered, this was noted and no further measurements were taken. Extended snow cover in both the upper and lower forest plots prevented full replicate measurements until late into the season.

In each of the five plots comprising each site, that were at least partially snow-free, twenty buds/leaves from the appropriate plant species were selected on different plants, using a random number table. If less than twenty different plants were present in the plots the buds/leaves were selected on different branches where possible. Each bud/leaf was carefully examined and categorized into four phases: bud, bud-burst, leaf visible or leaf unfurled. The term 'bud' was defined as the period when leaf buds were present prior to bud-burst. 'Bud-burst' was defined as the period when bud tissue swelled and some green tissue was evident. The term 'leaf visible' was defined as the period when the leaf shape was obvious but had not fully expanded. Finally, 'leaf unfurled' was defined as the period when the leaves were fully expanded, although not necessarily fully grown. If plots were still covered with snow when measurements were taking place it was assumed that all plants were in the bud phase. This assumption seemed reasonable

because in all cases plots recently released from snow cover contained plants only at the bud stage (pers. obs.).

After the phenology measurements were taken, the lengths of any fully unfurled leaves were measured by simply offering up the calliper tips to the leaf, thereby avoiding any unnecessary disturbance. When twenty or fewer unfurled leaves were present in a plot, all leaves were measured. When twenty or more unfurled leaves were present, twenty leaves were selected at random (on different plants or where species coverage was low on different branches) for measurement. The predicted area of each measured leaf was calculated using the regression equations given in section 2.4.

### 3.2.3 Data analysis

Statistical analyses were implemented in SPSS version 11 (SPSS Ltd., Chicago, Illinois, USA). Daily temperature data showed strong partial autocorrelation for the first six time lags. Because there was a risk of Type 1 error (i.e. wrongly rejecting the null hypothesis) due to artificially inflated degrees of freedom the data were re-sampled every six days.

For statistical analysis purposes dates were converted into days from 1 January 2003. Dates were also weighted for the proportion of leaves in each phenological stage and then compared using non parametric statistics.

## 3.3 Results

### 3.3.1 Air temperature

Air temperature was recorded at the four study sites located across the forest-tundra ecotone between 1 January and 14 June 2003. Mean air temperature across all sites was -3.5 °C (range: -32.2 °C to 12.7 °C; figure 3.1). Temperature patterns were strikingly similar across the ecotone with three distinct phases: an initial highly fluctuating phase where temperatures were well below freezing, a second phase where temperatures fluctuated around 0 °C and a third phase where temperatures increased and remained above 0 °C (figure 3.1).

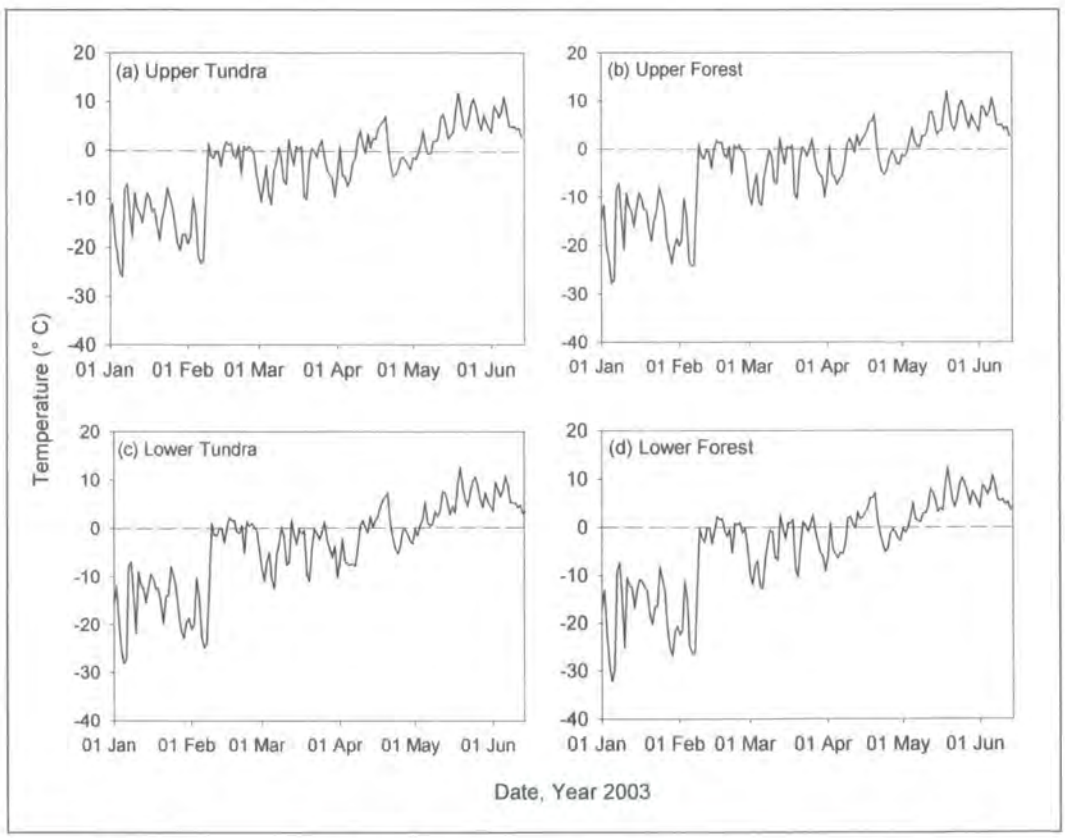


**Figure 3.1: Air temperature (°C) for (a) upper tundra, (b) upper forest, (c) lower tundra, and (d) lower forest.**

The shift between the first two phases of air temperature patterns was highly synchronized among the sites, occurring within two days at the start of

February. This shift was probably driven by insolation, as the rapid increase in air temperature in early February coincided with the sun reappearing over the horizon (figure 3.2).

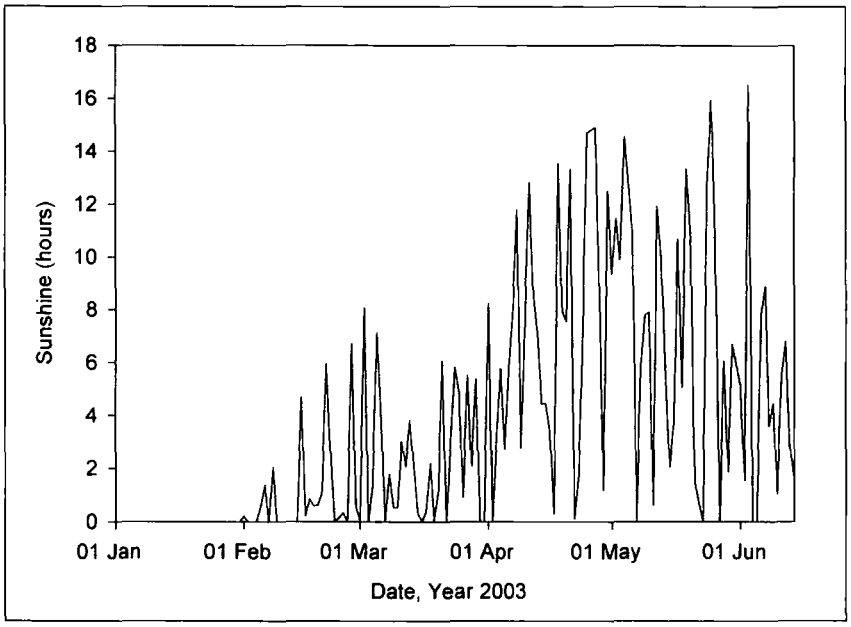


**Figure 3.2: Daily hours of sunshine recorded at ANS weather station. Mean 3.70 h of sunshine per day (range: 0 h - 16.52 h).**

A general linear model was constructed to investigate the influences of elevation and habitat on daily mean air temperature at the four sites. Date was entered into the model as a covariate to control for the effect of temperature generally increasing with season. The results of the model indicated that neither habitat nor elevation exerted a significant effect on air temperature across the ecotone transect (elevation: $F_{2,814} = 0.11$, $p > 0.8$; habitat: $F_{1,814} = 0.5$, $p > 0.4$). This result makes intuitive sense as the air temperature data loggers were located 2 m above the ground surface, well above the level of the insulating snow pack.

### 3.3.2 Soil surface temperature

Although air temperature profiles were similar across all sites, forest and tundra plots experienced strikingly different soil surface temperature regimes. Three distinct phases were again apparent at all sites: an initial phase where

temperatures fluctuated below 0 °C, a middle phase where temperatures were stable around 0 °C and a final phase where temperatures increased and remained above 0 °C (figure 3.3). During the initial phase, until about mid-February in forest and tundra plots, soil surface temperatures fluctuated greatly, suggesting that the snow cover was likely to be relatively thin particularly in the tundra plots, allowing air temperature to strongly influence the temperature at ground level. Mean air temperature at 2 m height during January was -15.6 °C, and this appeared to influence soil temperature at tundra sites far more than at the forest sites.

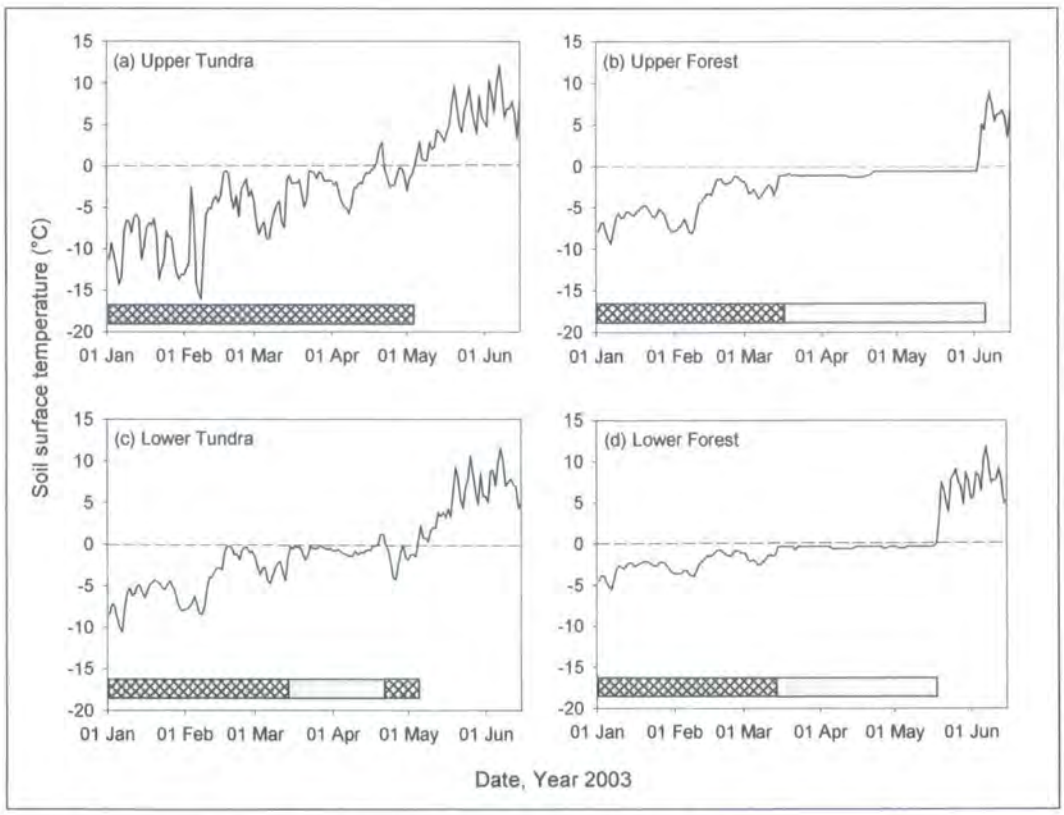


**Figure 3.3: Daily mean soil surface temperatures (° C) for (a) upper tundra, (b) upper forest, (c) lower tundra and (d) lower forest. The periods of definite snow cover are shown with grey bars while periods of uncertain snow cover are shown with hatched grey bars.**

The next phase in the pattern of soil surface temperature change was characterised by a long period of stable temperatures apparent in the forest plots (figure 3.3 (b) & (d)). The mean (± SD) soil surface temperature in lower forest was -0.36 °C ± 0.13 °C (range: -0.69 °C – 0.08 °C) between

16 March and 17 May. Similar soil surface temperatures were recorded in the upper forest sites where the mean (± SD) temperature was -0.84 °C ± 0.27 °C (range: -1.30 °C – -0.60 °C) between 16 March and 1 June. Such stable temperatures indicate that the soil surface was well insulated from ambient air temperatures by snow cover. In the tundra plots, however, relatively large fluctuations in soil surface temperatures continued throughout this period, particularly in the upper tundra plot, where temperatures ranged between -8 °C and 0 °C (figure 3.3 (a) & (c)), suggesting that the ground surface in tundra habitats was not insulated by snow cover.

The long period of stable temperatures recorded in both forest plots was probably associated with the pattern of snow lie in those sites. Although precipitation was unlikely to vary significantly among these sites (spanning a distance of only 1.9 km across the ecotone), wind speed in forested habitats is probably lower than in tundra habitats, thereby allowing a thicker snow pack to develop after snowfall. I tested this hypothesis by examining the pattern of soil surface temperature in relation to wind speed and precipitation. Soil surface temperatures in the forest plots stabilized in mid-March, just after a period of increased precipitation (figure 3.4 (a)), which is likely to have resulted in the formation of a dense isothermic snow pack. This dense snow pack appears to have formed only in the forested plots, something probably explained by the elevated wind speed also apparent around mid-March (figure 3.4 (b)). Wind speeds were at their highest level (approx 12 m/s) during this period of increased precipitation, suggesting that any snow lying on exposed sites was redistributed to lee areas and forests.

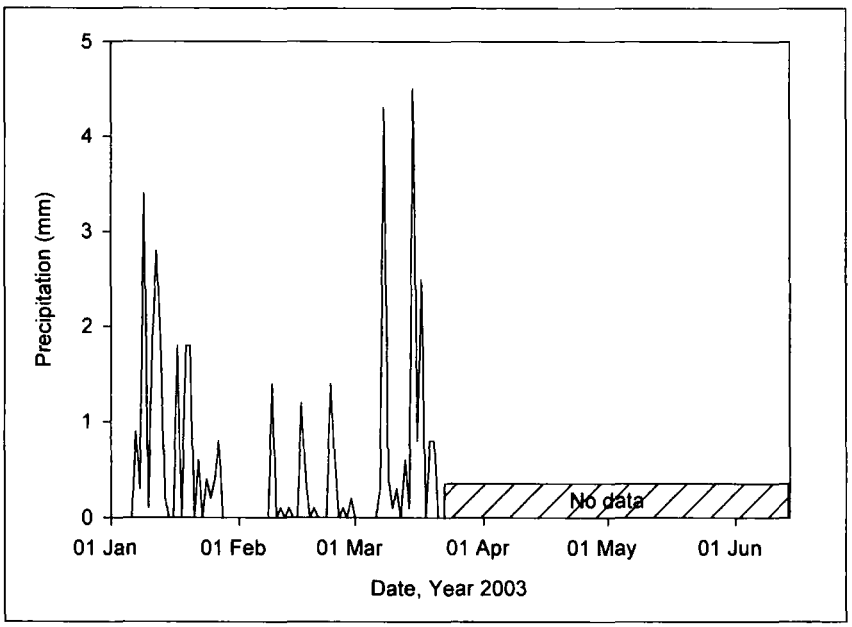


**Figure 3.4 (a): Daily precipitation (mm) recorded at ANS weather station, using a heated rain gauge. Mean daily precipitation was 0.36 mm (range: 0 mm – 4.50 mm). Highest precipitation levels were recorded immediately prior to the stabilizing of soil surface temperatures in early and mid-March. Technical problems prevented data collection between the 20 March and 14 June.**

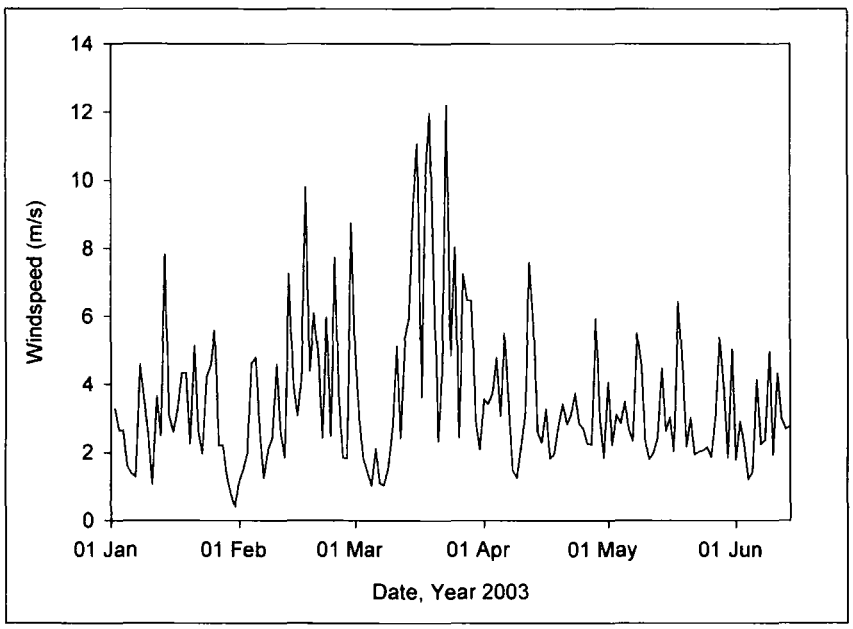


**Figure 3.4 (b): Daily wind speed (m/s) recorded at ANS weather station. Mean wind speed was 3.56 m/s (range: 0.41 m/s – 12.2 m/s). Highest wind speeds were recorded during period of highest precipitation (early and mid-March), probably affecting snow distribution on exposed tundra sites.**

The third phase of soil surface temperature patterns was characterised by temperatures rising and remaining above 0 °C. Direct field observations at the two forest sites showed that plots became snow-free on the same day that temperatures recorded at the soil surface rose and remained above 0 °C (plots became snow-free in the lower forest site on 18 May and in the upper forest site on 1 June; see figure 3.3 (b) & (d) for data from the temperature loggers). For the two tundra sites, the range of dates during which the plots were known to have become snow-free are shown on figure 3.3 (a) & (c). For the purposes of further analyses, it was assumed that the tundra sites became snow-free on the day that soil surface temperatures finally rose above 0 °C (3 May for the upper tundra and 4 May for the lower tundra).

The date on which temperatures rose above 0 °C and plots were therefore assumed to be snow-free varied between habitats and to a lesser extent with elevation. On the exposed tundra sites both air and soil surface temperatures increased above 0 °C at the start of May. In contrast, soil surface temperatures did not rise above 0 °C on the sheltered forest sites until the end of May for the lower forest and the start of June for the upper forest. This delay was probably a direct consequence of the length of time required to melt the thick isothermic snow pack that is likely to have covered the forest sites (see section 3.3.2). Despite the fact that soil surface temperatures in the forested sites began to rise much later than in tundra sites, the gradient of the rise was much steeper, because ambient temperatures were already high when the snow melted. This also meant that in tundra sites, where snow cover melted early, plants could be subject to frost damage during periods of low temperature (e.g. towards the end of April on the lower tundra site, figure 3.3 (c)).

**Table 3.1: Minimum, maximum and mean soil surface temperatures (°C) with associated standard deviations for upper tundra, upper forest, lower tundra and lower forest (1 January 2003 – 14 June 2003).**

| Elevation | Habitat | Min temp (°C) | Max temp (°C) | Mean (°C) | S.D. |
|---|---|---|---|---|---|
| Upper | Tundra | -16.03 | 12.09 | -2.39 | 6.15 |
| Upper | Forest | -9.41 | 8.9 | -2.07 | 3.38 |
| Lower | Tundra | -10.60 | 11.53 | -0.87 | 4.72 |
| Lower | Forest | -5.53 | 11.83 | -0.04 | 3.58 |

Soil surface temperatures varied widely across different elevations and habitats (table 3.1). A general linear model was constructed to assess the relative influence of elevation and habitat on soil surface temperature. Date was entered into the model as a covariate to control for seasonal effects. The higher elevation sites had lower soil surface temperatures than the lower elevation sites ($F_{1,660} = 84.57$, $p < 0.001$, table 3.1). Furthermore, habitat also exerted a strong effect on soil surface temperature independent of elevation, with forested sites being warmer than tundra sites ($F_{1,660} = 9.72$, $p = 0.002$, table 3.1). The interaction between habitat and elevation was not significant ($F_{1,659} = 2.20$, $p = 0.139$), and was removed from the model. Therefore, as well as showing differences in the pattern of temperature change, as discussed above, the habitats also showed different temperature levels, with the sheltered forest sites being warmer than the exposed tundra sites.

### 3.3.3 Difference between air and soil temperatures

Temperatures at the soil surface appeared to be insulated against fluctuations in air temperature to varying extents depending on elevation and habitat (figure 3.5). However, since snow depth could not be measured directly, the extent of the insulation was determined from the difference between air and soil surface temperatures. The patterns of difference between air and soil surface temperatures were similar across all sites, although the degree of protection varied considerably. In the sheltered forest sites, particularly the lower forest, a difference of between 18 °C and 27 °C

was observed between air and soil surface temperatures (figure 3.5 (b) & (d)). In contrast, a difference of only 11 °C to 17 °C between soil surface and air temperatures was found on the exposed tundra sites (figure 3.5 (a) & (c)).

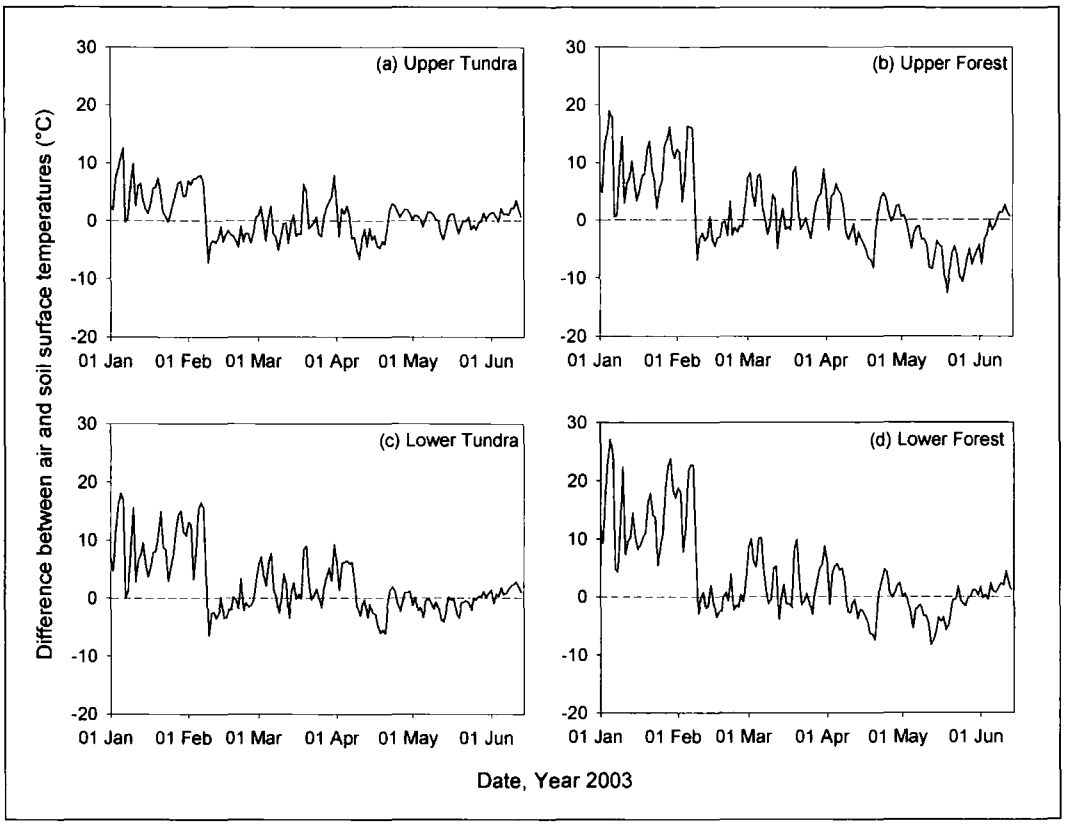


**Figure 3.5: Difference between soil surface temperatures (°C) and air temperatures (°C) at 2 m height for (a) upper tundra, (b) upper forest, (c) lower tundra, and (d) lower forest (calculation: soil temperatures – air temperatures). Positive values indicate that soil surface temperatures were warmer than air temperatures while negative values indicate that air temperatures were warmer than soil surface temperatures.**

A general linear model was constructed to determine whether the difference between soil and air temperatures was influenced by habitat and elevation. Date was entered into the model as a covariate to control for seasonal effects. There was a greater difference between air temperature and soil surface temperature at lower elevation sites than at higher elevation sites (lower sites difference of 3.11 °C between air and soil surface temperatures; upper sites difference of 1.07 °C between air and soil surface temperatures; $F_{1,652} = 28.25$, $p < 0.001$). Furthermore, the difference between air temperature and soil surface temperature was significantly greater in forest

sites than in tundra sites (forest sites difference of 2.55 °C between air and soil surface temperatures; tundra sites difference of 1.63 °C between air and soil surface temperatures; $F_{1,652} = 5.80$, $p = 0.016$), indicating that habitat exerted a strong effect on air and soil temperatures independent of elevation. As in the models described in sections 3.3.1 and 3.3.2 the interaction between habitat and elevation was not significant ($F_{1,651} = 0.53$, $p = 0.464$) and was therefore removed from the model. These results suggest that the sheltered sites (lower elevation, forested habitat) were more protected from fluctuations in temperature than the exposed sites (higher elevation, tundra habitat).

It is therefore evident that temperature regimes across the forest-tundra ecotone differed with respect to elevation and habitat. Forested sites at lower elevations were shown to have a higher degree of insulation from fluctuating air temperatures along with warmer soil surface temperatures. Although these conditions are favourable for protecting over-wintering plant and invertebrate species, the onset of the annual growth cycle was delayed due to the late melting of the snow pack, particularly in forested habitats. Given the differences in temperature regime associated with habitat structure, the present study also investigated the effects on the timing and development of leaf growth.

### 3.3.4 Plant phenology

#### 3.3.4.1 Phenological development of *Vaccinium myrtillus* and *Betula nana*

Over 90% of *V. myrtillus* leaves, at both higher and lower forest sites, were still in bud when measurements started at the end of May 2003 (figure 3.6 (a)). The onset of bud-burst in upper sites lagged approximately three weeks behind lower sites, although the rate of transition from the bud to bud-burst phase was equal at the two elevations (figure 3.6 (a)). The difference between the onset of leaf growth on *V. myrtillus* plants at upper and lower elevations was likely to be a direct result of the dense isothermic snow pack

that formed at the higher elevation forest site. It is not surprising, therefore, that the peak in the proportion of buds which had burst occurred 9 days earlier at lower elevations than higher elevations (figure 3.6 (b); mean date for bud-burst in lower forest = 5 June, mean date for bud-burst in upper forest = 14 June; $U_{277,180} = 8773.50$, $p < 0.001$). The plateau in the proportion of *V. myrtillus* buds that had burst at lower elevations was probably the result of variation in the date at which the plots within each forest site became snow-free. The altitudinal lag in leaf development is again evident in figure 3.6 (c), which shows clearly that leaf emergence occurred two weeks earlier at the lower elevation forest plots (mean date for leaf visible in lower forest = 10 June, mean date for leaf visible in upper forest = 24 June; $U_{177,50} = 238$, $p < 0.001$). Furthermore, leaves at higher elevations had only just begun to emerge when those at lower elevations were already beginning the final phase of their development, unfurling (figure 3.6 (d)).

Despite the fact that *V. myrtillus* plants in the lower altitudinal plots began their development about three weeks earlier than those in the higher elevation plots, by the end of the measurement period over 90% of the leaves at both elevations (100% for lower plots and 93% for upper plots) had completed their development. Plants subjected to a late start were developing much faster than plants which were released early from snow cover.

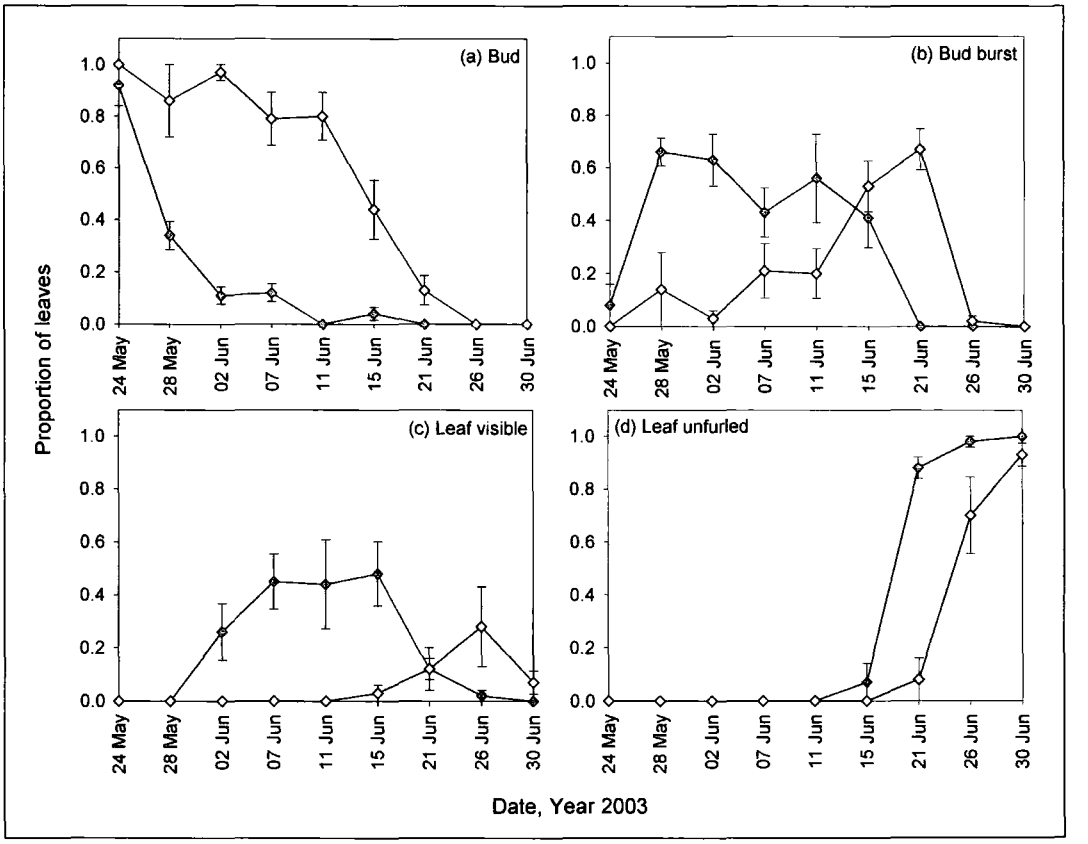


**Figure 3.6: Proportion of *V. myrtillus* (forest) leaves in (a) bud, (b) bud-burst, (c) leaf visible, (d) leaf unfurled phases of their development. Upper elevation sites are shown with open diamonds and lower elevation plots with filled diamonds. Error bars are ± 1 SE.**

*Betula nana* leaves began development earlier than *V. myrtillus* leaves; over 70% of *B. nana* buds had burst before measurements began on 24 May (figure 3.7 (a)). The early development of *B. nana* leaf tissue was associated with the early release from snow cover on the tundra sites as soon as air temperatures rose above 0 °C on 3 May (see section 3.3.1). The main period of bud-burst in the lower tundra site was about a week in advance of that in the upper tundra site (figure 3.7 (a)). However, during the final stages of leaf development (figure 3.7 (c) & (d)) *B. nana* plants at the two elevations were highly synchronised (mean date for leaf visible for lower tundra = 4 June, mean date for leaf visible for upper tundra = 5 June; $U_{289,255}$ = 35724.50, p = 0.531). These results indicate that both *V. myrtillus* and *B. nana* can alter their rate of development in response to changes in the length of the growing season.

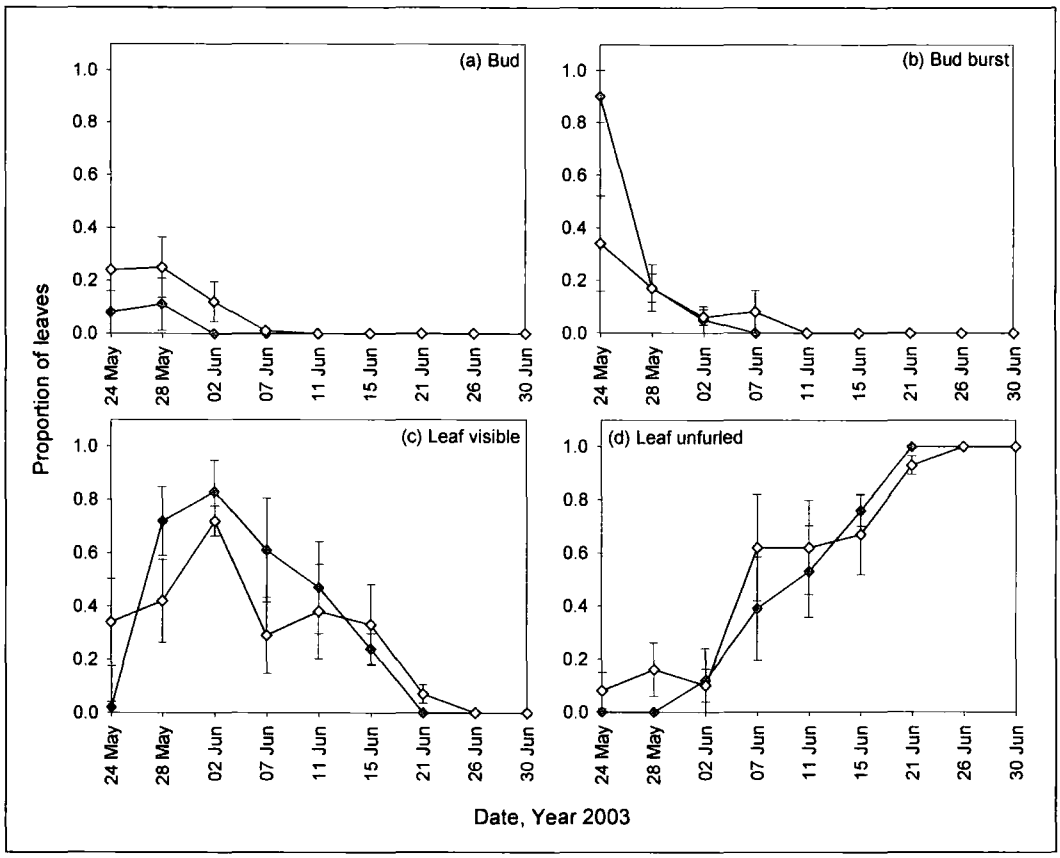


**Figure 3.7: Proportion of *B. nana* (tundra) leaves in (a) bud, (b) bud-burst, (c) leaf visible and (d) leaf unfurled phases of their development. Upper elevation plots are shown with open diamonds and lower elevation plots with filled diamonds. Error bars are ± 1 SE.**

### 3.3.4.2 Plant phenology in forest and tundra habitat

Between 24 May and 7 June there were significantly fewer shoots remaining at the bud phase on tundra plots than on forest plots (G-tests: all $p < 0.01$, figure 3.8 (a)). On the tundra plots, only 18% of the *B. nana* shoots remained at the bud phase on 24 May. This suggests that the majority of buds of tundra plants had burst before measurements began. In contrast, 98% of *V. myrtillus* plant shoots in forest plots were still in the bud phase on 24 May, suggesting that bud-burst had only just commenced when measurements began (figure 3.8 (b)). This probably reflects the extended snow cover observed in forested sites (see section 3.3.2).

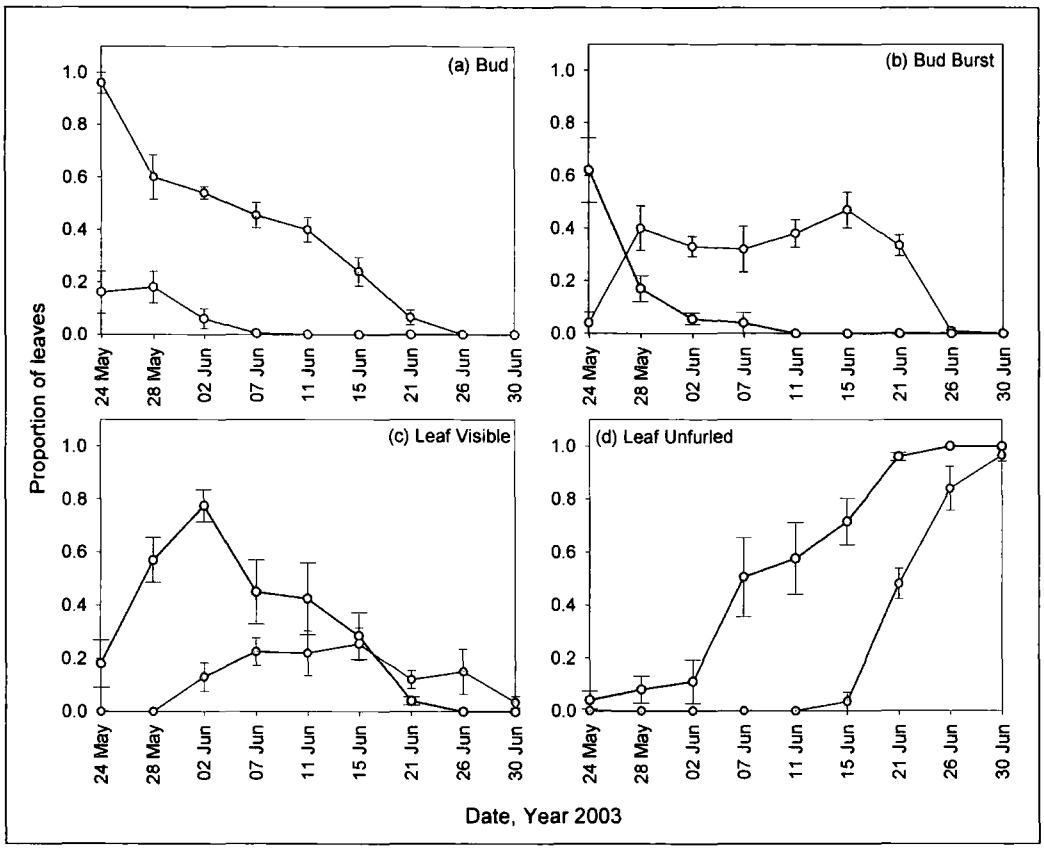


**Figure 3.8: Proportion of leaves in (a) bud, (b) burst-bud, (c) leaf visible and (d) leaf unfurled phases of their leaf development. Forest plots (*V. myrtillus*) are shown with filled circles and tundra plots (*B. nana*) with open circles. Error bars are ± 1 SE.**

The pattern of bud-burst for the forest plots showed a clear bimodality, peaking on 28 May and 15 June. The first of these maxima was caused by peak bud-burst in lower forest plants on 28 May, and the second by a peak in bud-burst in upper forest plants on 15 June (see section 3.3.4.1). Significantly more buds had burst in the forest plots than in the tundra plots between 28 May and 7 June (G-tests: all $p < 0.01$, figure 3.8 (b)). In the tundra plots, all buds had burst by 11 June, while in the forest plots, bud-burst was not complete until 30 June, a difference of approximately two and a half weeks.

The emergence of leaves from the buds occurred much earlier in tundra sites than in forest sites (figure 3.8 (c); mean date for leaf visible for tundra = 4 June, mean date for leaf visible for forest = 13 June; $U_{115,274} = 6049.50$, $p < 0.001$). The proportion of buds in the leaf visible phase peaked on

2 June in the tundra plots, but not until 15 June in the forest plots. The forest plots showed a much lower peak of leaf emergence than the tundra plots (forest plots: 26% leaf visible on 15 June; tundra plots: 79% leaf visible on 2 June), suggesting that *V. myrtillus* plants in the forest plots progressed more slowly between the leaf visible and unfurled phase. In the tundra plots, all *B. nana* leaves were fully visible by 26 June. In the forest plots, leaf emergence was almost complete by 30 June. These data indicate that, although the development of buds in forest plots was about 2.5 weeks behind bud development in tundra plots at the bursting phase, by the time leaf emergence was complete the gap had reduced to less than a week. Plants in forest plots appeared therefore to be compensating for their 'late start' by rapid bud development.

Data from unfurling leaf tissue supports the idea that late starting plants were developing at a faster rate than early starting plants (figure 3.8 (d)). Some fully unfurled leaves were observed in the tundra plots on 24 May, while the first unfurled leaves in the forest plots appeared only on 15 June, a gap of approximately three weeks. The rate of increase in the proportion of buds that had completed their development was much higher in forest plots than in tundra plots, again suggesting compensatory catch-up development by plants in the former habitat. All buds fully completed their development in both habitats on 30 June.

The plant phenology data demonstrate that leaf development varied considerably between forest and tundra habitats (figure 3.8). Although such differences may be at least partially the result of different emergence patterns between the two species measured, this study is primarily interested in when leaf material become available rather than how it does so. Bud development started much earlier in tundra plots than forest plots, although the transition between each phase of bud growth progressed more slowly in tundra plots (see section 3.2.2 for definitions). Plants in forested habitats showed very rapid leaf development, although bud-burst did not commence until relatively late in the season. Despite the different development patterns in forest and tundra sites, the date at which 100% of the leaves had reached

their final developmental phase (unfurled) was very similar for the two habitats (figure 3.8 (d)).

### 3.3.4.3 Effects of elevation on plant phenology

Considering *B. nana* and *V. myrtillus* together, elevation had a weaker, though still significant, effect on plant phenology (figure 3.9). On 24 May there were significantly fewer shoots remaining at the bud phase in lower elevations than higher elevations ($G_{adj} = 5.84$, d.f. = 1, $p < 0.02$). Despite this difference at the onset of leaf development, all buds in both the upper and lower sites had burst by 21 June and 26 June respectively.

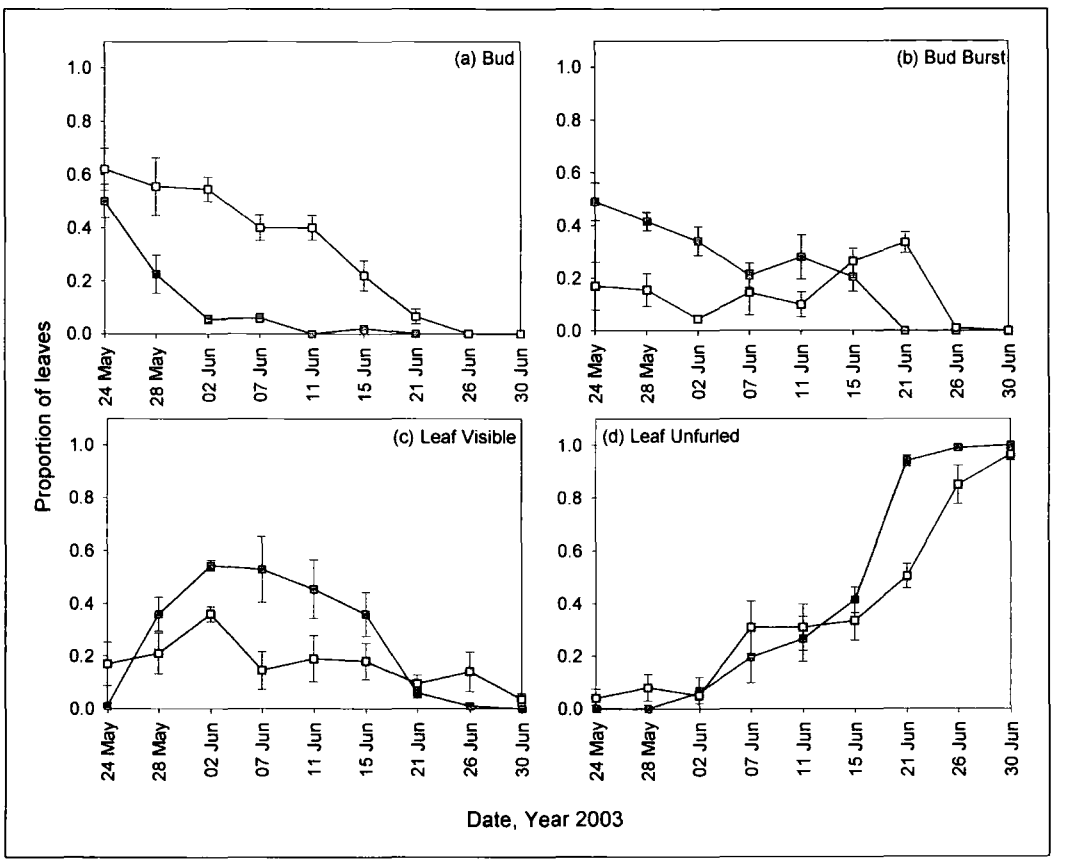


**Figure 3.9: Proportion of leaves in (a) bud, (b) bud-burst, (c) leaf visible and (d) unfurled phases of their leaf development. Lower elevation sites (lower forest and lower tundra) are shown with filled squares and upper elevation sites (upper forest and upper tundra) with open squares. Error bars are ± 1 SE.**

Bud-burst appears to have started early in the season with 50% of buds at lower elevations having burst, while only 19% had burst at higher elevations

by 24 May ($G_{adj}$ = 47.6, d.f. = 1, $p < 0.001$, figure 3.9 (a)). Figure 3.9 (b) clearly shows that the peak in the number of shoots that had burst at lower elevations occurred on or before 24 May. In contrast, this peak did not occur until 21 June at higher elevations. All buds at lower elevations had burst by 21 June approximately four days before bud-burst was complete at higher elevations (26 June).

The emergence of leaves from buds occurred slightly earlier in the higher elevation sites (24 May: 19% leaves visible) than lower elevations (24 May: 2% visible) ($G_{adj}$ = 36.76, d.f. = 1, $p < 0.001$). However, this difference was reversed as leaf emergence was completed approximately four days earlier than at the upper sites (lower sites completed by 26 June; upper sites almost complete by 30 June; figure 3.9 (c)). Despite this variability there was no significant variation in leaf emergence rates at different elevations ($U_{154, 234}$ = 17966, $p = 0.961$). A similar emergence pattern was observed during the final phases of leaf development, with leaves at lower elevations being fully unfurled by 26 June approximately four days before leaves at higher elevations (30 June; figure 3.9 (d)).

Although elevation did not appear to have a strong effect on plant phenological development, the delay between the completion of each phase at different elevations is still worth noting. In general, plants at lower elevation sites were slightly in advance of plants at upper elevation sites, by approximately one week.

### 3.3.5 Leaf growth

Leaf growth in tundra habitats (*B. nana*) was distinctly in advance of leaf growth in forest habitats (*V. myrtillus*, figure 3.10 (a), (c), (d)). Leaf growth on *B. nana* plants, at both upper and lower tundra sites, increased rapidly from 2 June until the end of the measurement period (30 June; figure 3.10 (a), (b), (d)). The closely matched patterns of *B. nana* leaf growth were expected as plots at upper and lower elevations were either snow-free throughout or became snow-free as soon as temperatures rose above 0 °C, thereby

allowing plants to use the full growing season. However, in forest habitats (*V. myrtillus*) leaf tissue growth did not commence properly until 15 June at lower elevations and 21 June at higher elevations (figure 3.10 (b)). Therefore, the growing season length for forest plants was reduced by approximately two weeks in comparison with tundra plants and by approximately one week in upper than lower forest sites. These lags were directly related to the prolonged snow cover at both the forest sites (see section 3.3.2). However, despite a difference of almost two weeks in the initiation of growth, the total leaf area available to herbivores in both forest and tundra habitats and at both upper and lower elevations were similar by the end of the measurement period (figure 3.10). Therefore, as *V. myrtillus* leaves attainted approximately the same total area as *B. nana* leaves it is clear that the former plant is capable of shifting its developmental rate depending on the length of the growing season. Further support for this idea came from the fact that *V. myrtillus* leaves at higher elevations reached the same size as those at lower elevations despite a delay of approximately one week in the onset of leaf growth.

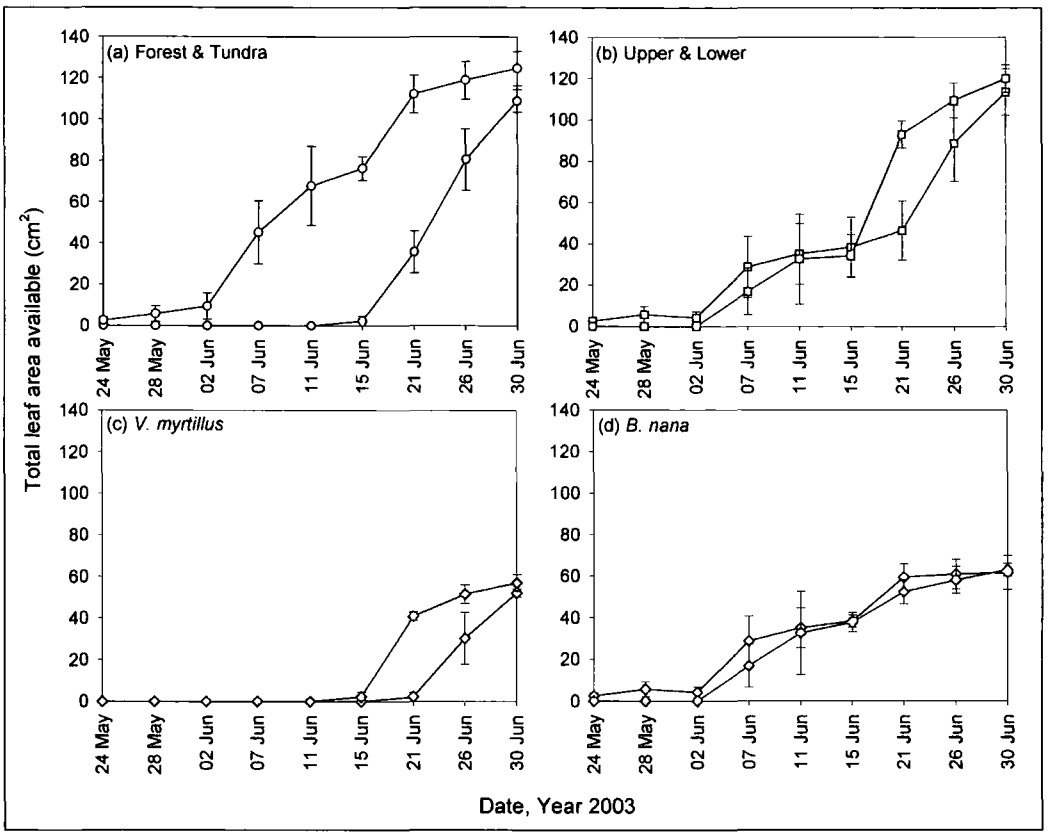


**Figure 3.10: Total leaf area available ($cm^2$) in (a) forest (*V. myrtillus*) (filled circles) and tundra (*B. nana*) (open circles) habitats; (b) upper (open squares) and lower (filled squares) elevations; (c) *V. myrtillus* at upper (open diamonds) and lower (filled diamonds) elevations, (d) *B. nana* at upper (open diamonds) and lower (filled diamonds) elevations. Total leaf area was calculated by averaging leaf area recorded in each session and multiplying by the number of unfurled leaves in each habitat at each session. Error bars are ± 1 SE.**

In terms of availability of leaf tissue to herbivores, it is evident that plant phenological development and leaf growth were affected by habitat and to a lesser extent elevation. Leaf development started later in forested habitats, but the development of tissue was accelerated in comparison with tundra habitats, suggesting that plants were compensating for their 'late start'. Furthermore, leaves at lower elevations started and completed their leaf development slightly earlier than those at higher elevations.

## 3.4 Discussion

The time available for leaf expansion across the forest-tundra ecotone was strongly dependent on snow melt patterns. As predicted, snow was present in both upper and lower forest sites for longer than in either of the tundra sites. Despite this delay, by the end of the measurement period, total leaf area in the sheltered forested areas was similar to the total leaf area recorded in the exposed tundra areas. Plants within the forest plots therefore appeared to be compensating for their late start by growing at a faster rate.

Air temperature regimes were broadly similar across the forest-tundra ecotone suggesting that local climates were not strongly influenced by topographic features (Danks, 1991). Air temperatures at all sites were, however, influenced by insolation as temperature ranges at the different sites were reduced when the sun reappeared over the horizon in mid-February. In contrast, soil surface temperatures were significantly warmer in the sheltered forest sites than in the exposed tundra sites. This pattern is likely to be directly related to the physical structure of the forest and tundra habitats and their ability to trap drifting snow. Soil surface temperature measurements and personal observations showed that snow was present in the forest areas even after leaf expansion had commenced on the tundra sites, confirming that prolonged snow cover reduces the length of the growing season (Billings & Bliss, 1959; Billings, 1987; Price & Waser, 1998; Price & Waser, 2000).

The phenological development of leaves on tundra sites was assumed to have started when air and soil surface temperatures rose above 0 °C (Sorensen, 1941). As snow cover in these areas was probably limited as a result of the persistent wind, plants would have had access to a full growing season. However, the plants will have been poorly protected from sub-zero air temperatures as well as being subjected to desiccation and abrasion from wind blown snow crystals (Sonesson & Callaghan, 1991). In contrast, the growing season for plants in the forested plots was limited by prolonged snow cover as growth could only commence once plots were free of snow.

These plants, however, will have been protected from most periods of sub-zero winter temperatures, desiccation, abrasion and summer drought (Billings & Bliss, 1959). Winter temperatures are, however, practically never harmful to plants, even under a thin snow cover, since their aerial parts either die or are hardened to withstand low temperatures (Sonesson & Callaghan, 1991). *Vaccinium myrtillus* plants in forested areas appear to complete their phenological development at a faster rate than *B. nana* plants in tundra areas so as to use the remaining summer period effectively. Due to the large amount of resources put into rapid leaf expansion, plants are probably paying a cost in terms of leaf structure and composition. For example, plants may be trading-off production of defensive alkaloids in favour of rapid growth (Herms & Mattson, 1992).

Results from phenological measurements in forest and tundra habitats showed that bud-burst occurred approximately two and a half weeks earlier in *B. nana* plants on tundra sites when compared to *V. myrtillus* plants in forest sites. However, this difference in phenological development was no longer apparent once leaves were fully expanded suggesting that leaf expansion of *V. myrtillus* in forest sites occurred at an increased rate to that of *B. nana* in tundra sites. Similar patterns were recorded by Galen & Stanton (1995) in several alpine plant species, including *Poa alpina* and *Sibbaldia procumbens*, where plants located in late melting areas had more rapid rates of leaf expansion. However, Wijk (1986) showed that the rate of leaf expansion in *Salix herbacea* was not altered in late melt areas. It is therefore evident that compensatory growth does not occur in all Arctic or alpine plant species. This difference may be related to different life history strategies. Leaf expansion in annual species could be expected to occur at an increased rate as their life cycle must be completed in a specified time period. In contrast, leaf expansion for perennial species might occur at a slower rate because defences may be more important than rapid growth rates. In either case, the resulting production rates for species whose growth rates are not altered by prolonged snow cover will undoubtedly affect the importance of the species in alpine and Arctic habitats (Billings & Bliss, 1959; Ehleringer & Forseth, 1980; Miller, 1982; Galen & Stanton, 1993).

In contrast to habitat, the effect of elevation on the phenological development of leaves was much more subtle. Plants at lower elevations were slightly more phenologically advanced than those at higher elevations. Billings and Bliss (1959), however, found that there was no significant difference in the rate of development of plants at different elevations. This pattern of leaf development is likely to have occurred as a result of soil-surface temperatures being significantly warmer in lower sites than higher sites.

Optimum plant growth is likely to occur in either forest or tundra habitats at lower elevations. Although leaf development commenced earlier in tundra sites the results clearly demonstrate that leaf expansion in forest sites occurred at a faster rate and therefore leaf development in both habitats was completed at approximately the same time. It is unclear from this study whether the faster rate of leaf development in forest sites was due to *V. myrtillus* developing more quickly than *B. nana,* or by habitat structure affecting plant growth patterns through modified temperature profiles. Further work should investigate the growth patterns of a single species in forest and tundra habitats to distinguish between these two possibilities. *Betula nana* occurs sparsely in forested habitats (pers obs.), so it should be possible to compare leaf development rates in this species in the two different habitats.

Finally, these results have important implications for the associated herbivores as emergence time must be closely synchronized to the development time of their food source. In other words, herbivores on tundra sites have a wider window for emergence when leaves are still in the early phases of development than herbivores in forest sites. However, as phenological development rates are greatly increased in forest plants it may mean that leaf defences against herbivores are reduced therefore making leaves more palatable to the invertebrates (Herms & Mattson, 1992). It would be interesting to determine whether plant chemical defences are affected by compensatory growth in this system.

# Chapter 4 – Natural variation in the timing and extent of invertebrate herbivory

## 4.1 Introduction

Close synchronisation between larval eclosion and bud-burst of the primary food plant has been demonstrated many times (Dixon, 1976; Wint, 1983; Cannell & Smith, 1986; Murray et al., 1989; Tikkanen et al., 1999; Klemola et al., 2003). However, little is known about variation in synchrony over small spatial scales, which could be substantial, especially across an ecotone, where habitat structure changes dramatically over relatively short distances. This study investigated the variation in herbivore-plant synchrony by studying the lepidopteran *Epirrita autumnata* and its primary food plants across the forest-tundra ecotone in northern Sweden. In the past, outbreaks of *E. autumnata* have resulted in the almost complete defoliation of mountain birch forests in the Abisko valley of northern Sweden (Tenow, 1996; Tenow & Bylund, 2000).

Like many other herbivorous insects, the larvae of *E. autumnata* feed preferentially on immature foliage. They must therefore ensure that their development is closely synchronised with that of their host plants (Klemola et al., 2003). If egg hatch occurs in advance of bud-burst on deciduous hosts, the risk of mortality increases because larvae must wait for fresh foliage to become available before they can begin feeding (Feeny, 1970; Wint, 1983; Watt & McFarlane, 1991; Hunter, 1992; Quiring, 1994; Hunter & Elkinton, 2000). On the other hand if hatching occurs well after bud-burst, the larvae having missed the opportunity to feed on highly nutritious juvenile leaves, will be forced to take mature leaves that have high levels of tannins and other defensive chemicals (Feeny, 1970; Shaw, 1984; Ayres & MacLean, 1987; Watt & McFarlane, 1991; Tikkanen & Lyytikainen-Saarenmaa, 2002; Klemola et al., 2003). Furthermore, owing to the lower nutritive quality of mature leaf tissue, larval growth rates will be reduced, increasing their susceptibility to parasitism and predation, and decreasing their reproductive success, and

ultimately their fitness (Virtanen & Neuvonen, 1999; Lill & Marquis, 2001; Watt & McFarlane, 2002; Tanhuanpaa et al., 2003). Indeed, in an experiment on sycamore aphids (*Drepanosiphum platanoidis* Schrank), Dixon (1976) showed that individuals that hatched at the time of bud-burst were fitter than those that hatched earlier or later.

Since there is much variability within and among years in the timing of bud-burst and egg hatching (Danks, 1992; Aizen & Patterson, 1995; Dixon, 2003), how do insects ensure they develop within the optimal phenological window allowing feeding on immature leaves? Perhaps the safest method for ensuring optimal developmental conditions is for egg hatch to be controlled directly by the physiological status of the host plant (Harrington et al., 1999), although there is scant evidence of this occurring in nature (Leather et al., 1993) because egg development has to commence long before bud-burst occurs. Alternatively, herbivores could initiate hatching using the same physical cues that plants use to initiate growth. Indeed, several studies have demonstrated that the timing of both bud-burst and egg hatching are a function of the intensity of winter chilling and subsequent spring temperatures (Cannell & Smith, 1986; Murray et al., 1989; Leather et al., 1993).

The mechanisms of responses to temperature for both plants and insects, however, need not be identical to maintain developmental synchrony, not least because over-wintering stages of plants and insects may be subject to radically different temperature profiles. As a result, insects have evolved different developmental thresholds, degree-day requirements and winter chilling requirements from those of their hosts (Watt & McFarlane, 2002). Furthermore, the cues regulating the timing of egg hatch appear to be inherited (Komatsu & Akimoto, 1995). This implies that if climate change significantly altered the timing of bud-burst, any surviving herbivores would be individuals with inherited responses enabling them to track the phenology of their host plant more closely. As these individuals are more likely to mate, significantly more of the population will hatch in synchrony with bud-burst in the following year. Therefore, strong selective pressure should maintain

close synchrony between eggs and buds ensuring that herbivores develop within the optimal phenological window. Indeed, selection pressure can generate synchronisation at a very fine scale, for example insect egg hatching can be synchronised with bud-burst of individual trees (Mopper & Simberloff, 1995; Van Dongen et al., 1997). A possible exception to this is when the underlying mechanism governing herbivore phenology becomes redundant, for example where climate warming removes the winter chilling element required to stimulate bud development (Dewar & Watt, 1992). Under these circumstances, herbivore populations could easily be driven to extinction.

If strong stabilising selection is responsible for ensuring synchrony between invertebrate herbivores and their host plants, there could be variation in the degree of phenological synchrony over short spatial scales. For example, the present study has shown that the optimal feeding window for Arctic herbivores (i.e. high availability of developing tissue) is wider in tundra habitats than nearby forest habitats (see chapter 3). This, therefore, provides an ideal system to investigate local variation in the degree of phenological synchrony between herbivores and their host plants.

### 4.1.1 Hypotheses

In situations where leaf development occurs over an extended period (i.e. a relatively low proportion of plants with immature palatable leaf tissue at any one time), the emergence window for herbivores will be wider, and one might predict lower phenological synchrony. To test this hypothesis, leaf development under three different sets of environmental conditions was measured, although first the effect of defensive buds scales on some food plant species had to be considered.

#### 4.1.1.1 Bud defence and invertebrate herbivory

Unlike *Vaccinium myrtillus*, the buds of *Betula* spp. are covered by tough scales making them impenetrable to invertebrate larvae (Klemola et al.,

2003). Therefore, even though bud-burst occurs earlier in *Betula* spp., herbivory would not be expected to commence until these defences were removed (i.e. when leaves began to unfurl). Since *V. myrtillus* buds have no bud defences they were predicted to be subjected to herbivory from an earlier stage of their development (i.e. even before leaf flush).

The timing of the onset of herbivory in relation to plant phenology was compared in three different environmental conditions. The first location (tundra sites) was in open tundra habitat where the snow layer was dispersed early in the season. In the tundra sites, where *Betula nana* was the main food plant of *E. autumnata*, leaf emergence commenced in mid-May (see section 3.3.4.1). The second location (lake-forest site) was a low altitude birch forest (360 m) where a thick layer of snow accumulated through the winter but melted early in the season. In a similar manner to those in tundra sites, unfurling of *Betula* leaves (in this case *Betula pubescens* ssp. *tortuosa*) commenced at the beginning of June. The final location (forest sites) was a birch canopy-covered ground flora that included *Vaccinium myrtillus* and was protected from the harsh late winter weather by a deep, long lying, isothermic snow pack. Since the snow pack frequently persisted until mid-late May (Huntley, 2002) the onset of leaf development was delayed relative to the other sites, but because *V. myrtillus* buds were not protected by impenetrable scales, plant tissue would be available to herbivores as soon as buds had swollen.

The present study therefore tested the hypothesis that in *Betula* plants, herbivory would only commence when leaves began to unfurl, but in *V. myrtillus*, herbivory would commence when most leaves where still in the bud stage.

#### 4.1.1.2 Synchrony between plant species and their associated herbivores

To investigate whether synchrony varied with growing season length, the present study compared the patterns of herbivory under different

environmental conditions. As discussed above, both the tundra and lake-forest sites had relatively long growing seasons while the onset of growth in the forest sites was delayed by the long-laying snow cover. Therefore, in the tundra and lake-forest sites, the degree of synchronisation between leaf growth and the onset of herbivory is predicted to be relatively low as there is a long window of leaf growth. By contrast, in the forest sites, the window of fresh leaf growth was much narrower because of the shorter growing season length compounded by the effect of compensatory growth. Therefore, in the forest sites, high levels of synchrony between plants and their associated herbivores would be expected. The total impact of herbivory may, however, be relatively low as leaf growth is rapid and herbivores that are not in synchrony will be at a disadvantage.

The present study therefore tested the hypothesis that synchrony between herbivores and the host plants will be directly influenced by the length of the growing season, i.e. highly synchronised in forest sites when there is a short window of available leaf tissue, and less synchronous in tundra and lake-forest sites.

## 4.2 Methods

### 4.2.1 Phenology of deciduous leaves

The phenological development of leaves was recorded between the end of May 2003 and beginning of July 2003 for *B. nana* in tundra sites, *V. myrtillus* in forest sites and *B. pubescens* ssp. *tortuosa* in the lake-forest site (see section 2.1 for descriptions of the sites). The plots were monitored approximately every four days, although the prolonged snow cover on forest sites prevented full access to the plots until relatively late in the season. The developmental stage of twenty randomly-chosen bud sites in each plot was recorded during each sampling session. For full details of the methods and terminology used to describe the phenological development of leaves in forest and tundra sites see section 3.2.2 (page 20).

The lake-forest site was situated in a forest patch close to the Abisko Scientific Research Station (68°21`N 18°49`E, Abisko Naturvetenskapliga Station, Abisko SE – 98107, Sweden). A single branch, approximately 1.5 m above ground, was selected on nine randomly chosen *B. pubescens* ssp. *tortuosa* trees. On each branch, fifteen bud sites (5 bud sites closest to the trunk, 10 bud sites closest to the branch tip) were marked with a piece of cotton and a small piece of tape with a unique identification number to facilitate subsequent identification. Measurements of bud site phenology and leaf growth were taken every two days between 24 May and 30 June. In each sampling session a further five buds/leaves were randomly selected, using a random number table, along the branch immediately below each marked branch. During each sampling session, the buds/leaves were categorised into one of four developmental phases namely bud, bud-burst, leaf visible and leaf unfurled (see section 3.2.2, page 20, for definitions).

### 4.2.2 Deciduous leaf area available to herbivores

During each sampling session, twenty fully unfurled leaves were selected, using a random number table, on different shrubs in five 1 $m^2$ plots in forest (*V. myrtillus)* and tundra (*B. nana*) habitats at upper and lower elevations. Using digital callipers, the length of each leaf was measured from the tip of the petiole to the tip of the leaf (or estimated position of the tip if the distal portion of the leaf had been removed through herbivory). Similar length measurements were taken for the selected *B. pubescens* ssp. *tortuosa* leaves (see section 4.2.1).

The predicted area of each measured leaf in the absence of herbivory was calculated using the regression equations given in section 2.4 (page 11). Mean leaf area during each sampling session was multiplied by the number of fully unfurled leaves to provide an index of the relative area of leaf tissue available to herbivores in each of the plots. Measurements were not taken directly from the leaves scored for phenology because these would have provided an inadequate sample size, particularly early in the season when few leaves were fully unfurled.

### 4.2.3 Determining deciduous leaf area removed by herbivores

To estimate the proportion of leaves that had been attacked by herbivores, twenty fully unfurled leaves were randomly selected and examined for signs of herbivory. When twenty or fewer unfurled leaves were present in a plot all leaves were measured. When more than twenty such leaves were present, twenty were selected at random for measurement. For leaves where herbivory had occurred, leaf length was measured, and the predicted total area of the leaf calculated as described in section 4.2.2. To estimate the actual area of tissue removed by herbivory, diagrams of the damage on each leaf were recorded on standard pre-drawn templates of the leaves of each plant species (figure 4.1).

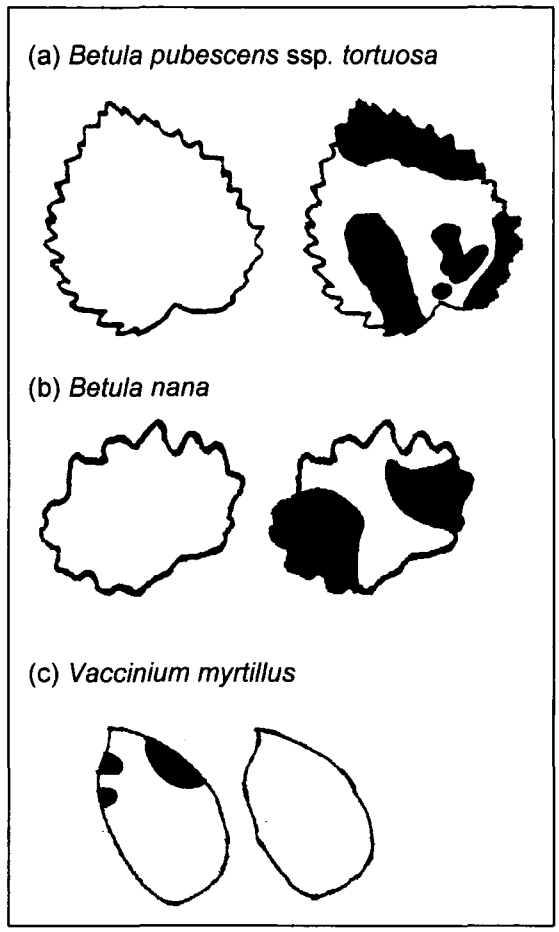


**Figure 4.1: Example of pre-drawn templates used to record invertebrate herbivory damage on (a) *B. pubescens* ssp. *tortuosa*, (b) *B. nana*, and (c) *V. myrtillus*.**

To determine the extent and development of herbivory damage on the leaves, the first twenty leaves in each plot that showed evidence of herbivory were marked with a piece of cotton and a small piece of tape with a unique identification number. On each subsequent visit, all marked leaves were re-measured and new diagrams of herbivory taken, irrespective of further random sampling of other leaves for phenological and area scoring.

In the lake-forest site, once each of the buds previously identified on the nine *B. pubescens* ssp. *tortuosa* trees had unfurled, a single leaf was randomly chosen from each bud site (more than one leaf always emerged from a single bud site). The other leaves in the bud site were marked with Tippex to allow for future identification. The target leaf was not marked in any way, to minimise disturbance to any herbivores present on the leaf, and great care was taken when manipulating leaves and branches for herbivory studies. During each sampling session, the length of each leaf was measured using digital callipers (see section 4.2.2). In a similar way to that for forest and tundra sites, templates were used to record and monitor evidence of herbivory on the leaves.

Completed templates for *B. pubescens* ssp. *tortuosa, B. nana* and *V. myrtillus* were digitised using a scanner at a resolution of 600 dpi, and analysed with the SigmaScan Pro software (SPSS, Chicago, Illinois, USA). The software provided measurements of the area of removed tissue on the template drawings, which were individually scaled to the size of each leaf using the 2-point rescaling calibration function within SigmaScan Pro. The actual area removed by invertebrate herbivory on each leaf was then calculated. Predicted total leaf area in the absence of herbivory was calculated by using the regression equations described in section 2.4 (page 11). For each leaf, therefore, estimates of original leaf area and the area removed by herbivory were obtained.

### 4.2.4 Evergreen herbivory

The extent of invertebrate herbivory on *Vaccinium vitis-idaea* was recorded in forest and tundra habitats at both upper and lower elevations. In each plot, individual plants were randomly selected using a random number table. All leaves on each plant were examined and the number of leaves showing evidence of herbivory was recorded. A leaf was defined as having been attacked by herbivores if a crescent of tissue had been removed. Leaves which were cleanly broken or that had black necrotised tissue were not included as these appeared to be the effects of frost damage. A new plant was selected at random in each plot and the measurements repeated until 10 leaves with evidence of herbivory were identified, a total of 500 leaves had been examined, or all plants in the plot had been counted. For statistical analyses, plants in plots covered by late melting snow were assumed to be 'protected' from invertebrate herbivores and therefore the proportion of leaves with herbivory was assumed to be the same as that for the first snow-free measurement period in the plot (i.e. any herbivory on leaves would have occurred during the previous season).

## 4.3 Results

### 4.3.1 Effects of habitat

#### 4.3.1.1 Herbivory start date and leaf phenological phase

Invertebrate herbivory patterns across the forest-tundra ecotone appeared to be influenced primarily by the date on which herbivory started. Herbivory in both the tundra and lake-forest sites commenced at approximately the same time: 11 June and 7 June respectively. When herbivory commenced in these sites, more than 90% of the leaves were in the final phases of their development – leaf visible and unfurled (table 4.1). In contrast, herbivory was not observed in forest sites until 21 June at which point over 70% of

leaves were still at the bud phase or only just beginning to burst (table 4.1). These results clearly indicate that on *Betula* spp., where the buds are protected by impenetrable scales, the onset of herbivory was delayed until the individual leaves had begun to emerge. However, the *V. myrtillus* buds do not have this defence mechanism, so invertebrate herbivores were able to attack the leaves at a much earlier stage in their development.

**Table 4.1: Percentage of leaves at different phenological phases at the onset of herbivory: 21 June for forest sites, 7 June for lake-forest site and 11 June for tundra sites. See section 3.2.2 for definitions of the different phenological phases.**

| Phenological phase | Forest site | Lake-forest site | Tundra site |
|---|---|---|---|
| Bud | 40 | 0 | 6 |
| Bud-burst | 38 | 0 | 5 |
| Leaf visible | 22 | 34 | 77 |
| Unfurled | 0 | 66 | 12 |

#### 4.3.1.2 Patterns of synchrony: Lake-forest site

The patterns of herbivory and leaf development were strikingly different across the three sites on the forest-tundra ecotone. Firstly, at the lake-forest site, food availability for herbivores on *B. pubescens* ssp. *tortuosa* trees, expressed as total leaf area, appeared to show two discrete phases of change, an initial rapid increase at the start of June and a second more gradual linear increase for the rest of the season (figure 4.2). The first rapid increase in leaf area probably represented the unfurling of existing leaf tissue, as 95% of *B. pubescens* ssp. *tortuosa* leaves unfurled between 2 June and 7 June. The second slower increase therefore probably represented the growth of unfurled tissue that stabilised toward the end of June, suggesting that leaf growth was complete by this time.

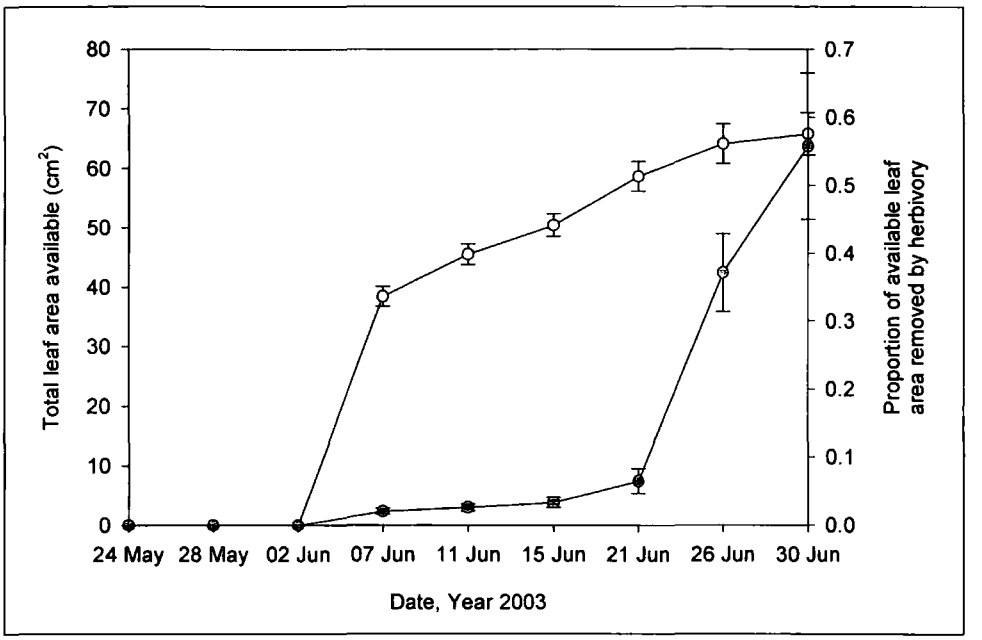


**Figure 4.2: Total leaf area available (open circles), and the proportion that had been removed by invertebrate herbivores in the forest-lake site (closed circles). Error bars area ± 1 SE.**

In contrast to food availability, total leaf area removed by herbivores increased very slowly until mid-June despite the fact that leaf tissue was readily available (figure 4.2). A rapid increase in the amount of tissue removed by herbivores was apparent in the second half of June. By the end of the measurement period, about 60% of total leaf tissue had been removed through herbivory. The time lag between the rapid increase in food availability and rapid assimilation of leaf tissue by herbivores was about three weeks.

#### 4.3.1.3 Patterns of synchrony: Tundra site

At the tundra sites, leaf tissue began to unfurl from the end of May, although there was a delay of approximately one week until the leaf area began to increase rapidly (figure 4.3). Herbivory, however, commenced and rose steadily from mid-June approximately two weeks after leaf tissue became readily available. By 26 June over 2.5% of available leaf tissue had been removed by herbivores. However, on the last measurement session, the

proportion of leaf tissue removed by herbivores appeared to drop to less that 1%. As the leaves appeared to be almost fully grown (figure 4.3), the decrease in the proportion of leaf tissue removed by herbivores is unlikely to have been caused by leaf tissue expansion. Therefore, the result is probably due to a chance sampling effect.

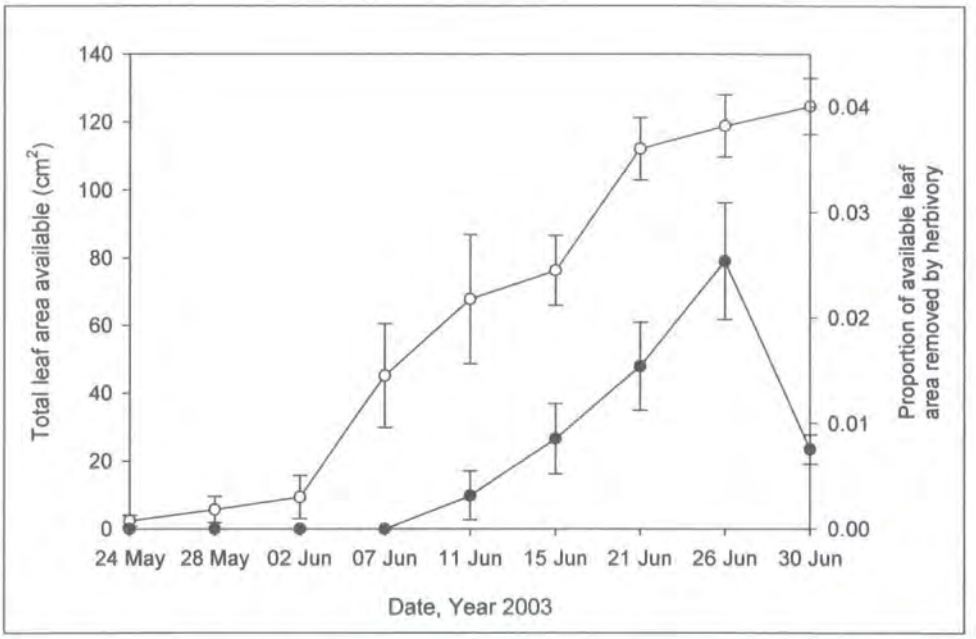


**Figure 4.3: Total leaf area available (open circles), and the proportion that had been removed by invertebrate herbivores in tundra sites (closed circles). Error bars are ± 1 SE.**

#### 4.3.1.4 Patterns of synchrony: Forest site

In contrast to the tundra and lake-forest sites, herbivores in forest sites appeared to be in close synchrony with the host plant, *V. myrtillus* (figure 4.4). In other words, herbivores started to consume the leaf tissue as soon as it became available. The slight drop in the proportion of leaf area removed by herbivores between 21 June and 26 June is likely to have been caused by the proportion of leaf area removed decreasing relative to the growing leaf area.

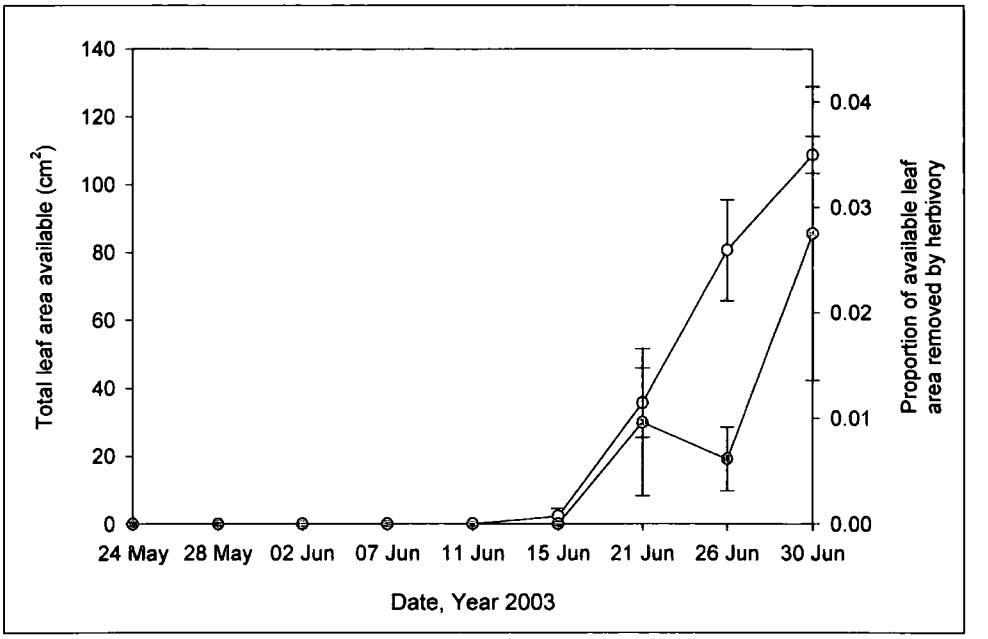


**Figure 4.4: Total leaf area available (open circles), and the proportion that had been removed by invertebrate herbivores in forest sites (closed circles). Error bars are ± 1 SE.**

By the end of the measurement period a larger proportion of available leaf tissue had been removed by herbivores in the lake-forest site (58% removed by 30 June) in comparison with the tundra (2.7% removed by 26 June) and forest (2.8% removed by 30 June) sites. Such a striking difference probably occurred because *B. pubescens* ssp. *tortuosa* is the main host plant for *E. autumnata* (Klemola et al., 2003). These results demonstrate that herbivores can still have a large impact on their host plant even when they show a relatively low level of developmental synchrony. However, the proportion of leaf material removed by herbivores is likely to be strongly influenced by the size of the larvae.

### 4.3.2 Effects of elevation

Herbivory patterns at different elevations were markedly similar despite a slight difference in start dates. On the lower sites herbivory did not commence until 15 June even though leaf material was readily available from 2 June. Comparable patterns of leaf availability were observed at higher

elevations although herbivory started slightly earlier (11 June). Furthermore, in direct contrast to the lower sites, the proportion of leaf material that was removed by herbivores on the upper sites levelled off and subsequently decreased slightly, from 21 June until the end of the measurement period (30 June), suggesting that the density of herbivores may have been very low. By the end of the measurement period twice the amount of leaf tissue had been removed by herbivores at lower sites than at upper sites, although this difference was not significant ($U_{10,10} = 30.5$, $p = 0.143$). The impact of herbivores across the forest-tundra ecotone therefore appeared to be influenced more by habitat type than elevation. This difference is probably a consequence of delayed start in leaf availability in forest sites due to prolonged snow cover in these habitats (see section 3.2.2).

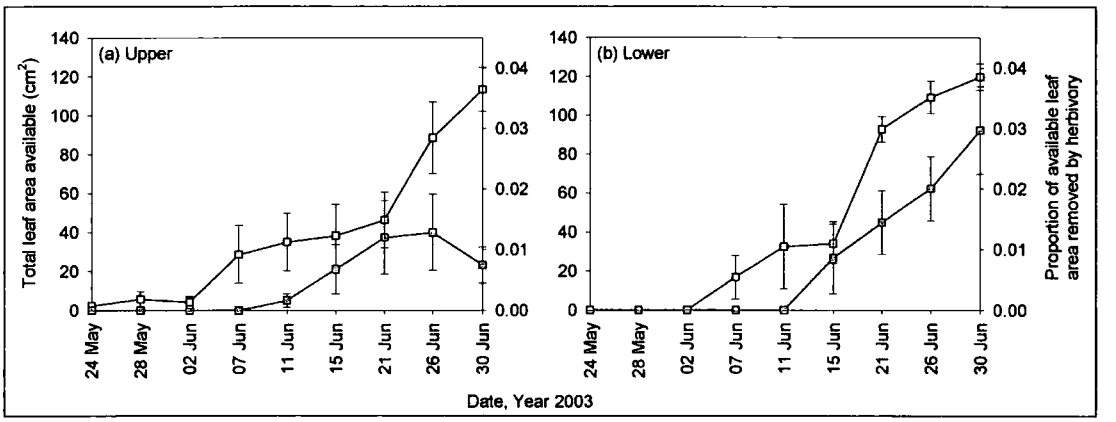


**Figure 4.5: Total leaf area available (open squares) and the proportion that had been removed by herbivores at (a) upper elevations and (b) lower elevations (closed squares). Error bars are ± 1 SE.**

### 4.3.3 Effects of plant life history strategy

Deciduous plants were subjected to greater levels of invertebrate herbivory than evergreen plants. In both forest and tundra habitats the proportion of leaves affected by herbivory increased rapidly, from 15 June and 7 June respectively, shortly after leaf material became available (see section 3.3.4; figure 4.6). In contrast, the proportion of evergreen leaves affected by herbivory remained very low throughout the measurement period with less than 3.2% of leaves being affected (figure 4.6). Importantly, the proportion of

evergreen leaves with herbivory did not increase with deciduous herbivory, confirming that deciduous leaves were preferred by herbivores.

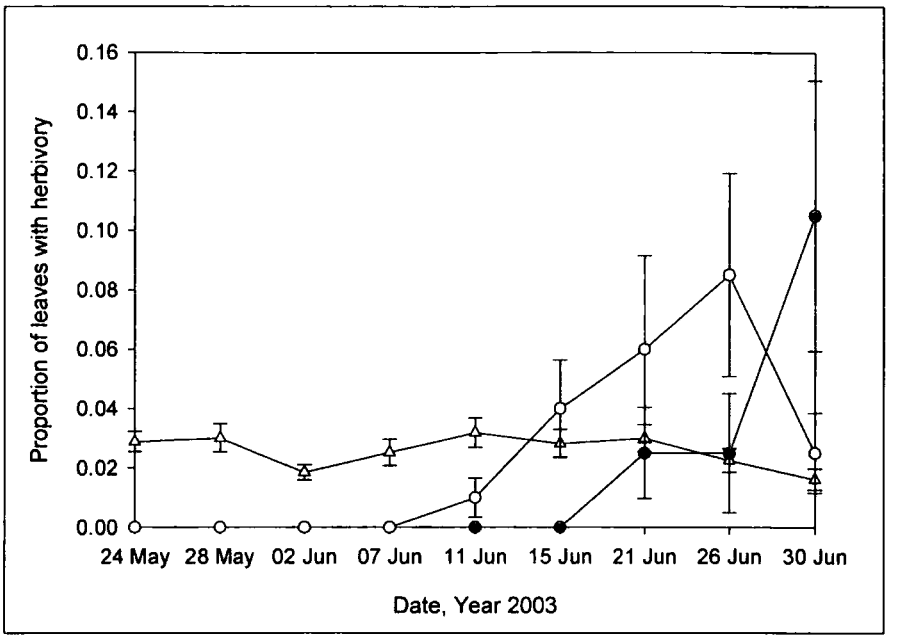


**Figure 4.6: Proportion of *V. vitis-idaea* (open triangles), *B. nana* (open circles) and *V. myrtillus* (closed circles) leaves with herbivore damage. Error bars are ± 1 SE.**

More *B. nana* leaves were attacked by invertebrate herbivores than *V. myrtillus* leaves (G-tests: all $p < 0.04$), except on 21 June ($G_{adj} = 0.50$, d.f. = 1, $p > 0.51$). This is likely to be a result of leaves of tundra plants emerging before leaves of forest plants. By the end of the measurement period approximately 10% of *V. myrtillus* leaves had been attacked by herbivores. In contrast, figure 4.6 shows that the proportion of *B. nana* leaves with herbivory decreased markedly in the last measurement period from 8.5% on 26 June to 2.5% on 30 June. The large standard error bars suggest that this may be due to chance sampling effects.

Although invertebrate herbivory on evergreen plants remained low across the measurement period, figure 4.7 (a) shows that more evergreen leaves were attacked by herbivores in forest plots than tundra plots (Wilcoxon: $Z = 2.67$, $n = 9$, $p = 0.008$). Despite this difference in amplitude, the patterns of evergreen herbivory in forest and tundra habitats were similar.

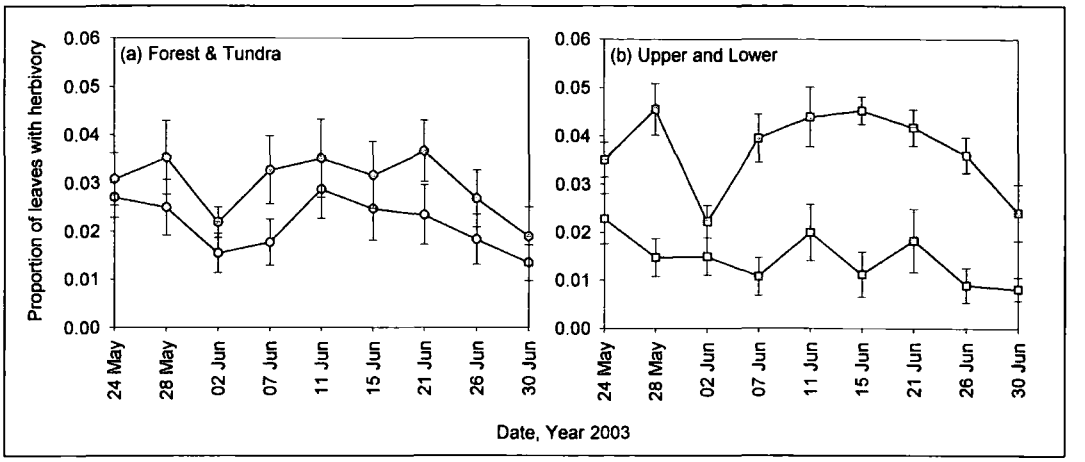


**Figure 4.7: Proportion of *V. vitis-idaea* leaves with herbivory in (a) forest (closed circles) and tundra (open circles) habitats; and at (b) lower (closed squares) and upper (open squares) elevations. Error bars are ± 1 SE.**

Furthermore, increased levels of evergreen herbivory were found at lower elevations than higher elevations (Wilcoxon: $z = 2.67$, $n = 9$, $p = 0.008$; figure 4.7 (b)). The percentage of leaves affected at higher elevations ranged between 0.9% and 2%, whereas at lower elevations the range was much higher at 2% to 4%, indicating that evergreen herbivory patterns differed markedly between elevations.

By the end of the study period, there was a greater frequency of 'deciduous herbivory' than 'evergreen herbivory' in tundra sites than forest sites ($G_{adj} = 3.72$, d.f. = 1, $p = 0.05$). Similar patterns were also observed at lower elevation sites than at higher elevation sites ($G_{adj} = 9.58$, d.f. = 1, $p = 0.01$). Evergreen plants therefore appeared to be subjected to relatively similar levels of herbivory in all habitats and at all elevations. In contrast, deciduous plants were at far greater risk of herbivory in tundra habitats and at lower elevations.

## 4.4 Discussion

### 4.4.1 Synchrony and habitat

Synchrony between herbivores and their host plants was much greater in the forest habitats than in the tundra and lake-forest habitats. This difference in synchrony is likely to be a direct result of the tough scales on birch buds making them impenetrable to herbivores (Klemola et al., 2003). Further analysis of the data confirms that herbivory commenced on *B. pubescens* ssp. *tortuosa* and *B. nana* when 90% of leaves were in the final phases of leaf emergence. Herbivores are therefore likely to have delayed their emergence to ensure that sufficient food supplies were available. Such an effect was, however, not observed on *V. myrtillus* since the leaves are poorly defended, allowing herbivores to attack them while still in the bud phase (over 70% of leaves were still in the bud phase when herbivory started in the forest plots). Therefore, although leaf development may occur more rapidly in forest plots herbivores are able to emerge at any stage of leaf development as a supply of food will always be available. However, herbivores that coincide with the younger leaves will be at an advantage as the tannin levels will be low while nitrogen levels will still be high (Moran & Hamilton, 1980; Shaw, 1984; Herms & Mattson, 1992).

The high level of protection adopted by *Betula* species was likely to have contributed to the lag that occurred between the onset of leaf growth and the start of herbivory in both the tundra and lake-forest sites. However, as leaf development on both *B. pubescens* ssp. *tortuosa* and *B. nana* commences early in the growing season herbivores with a delayed hatching date may be selected for, to ensure that weather conditions are favourable upon emergence. It has been well documented that few insect species are able to tolerate freezing without some form of protection (freeze-tolerance or freeze-avoidance by supercooling; (Strathdee & Bale, 1998; Bale, 2002) and therefore if larvae emerged when temperatures were fluctuating around 0°C the risk of death would be greatly increased. Certain areas of forest within

the Abisko valley have been protected from past defoliations as the eggs of *E. autumnata* were killed when temperatures fell below the supercooling point (Tenow & Nilssen, 1990; Tenow, 1996).

Finally, significantly more leaf material was removed from *B. pubescens* ssp. *tortuosa* plants than from either *B. nana* or *V. myrtillus*. To a certain extent such patterns were expected as *B. pubescens ssp. tortuosa* was the dominant host plant for the autumnal moth in the area (Tenow, 1996). However, as the forest and tundra sites were located at higher elevations it is possible that the growing season was slightly delayed. Therefore, after the measurement period both leaf area and the proportion of leaf tissues removed by herbivores might have continued to increase mirroring the lake-forest site. However, it is also possible that conditions in the higher sites were less favourable and therefore both leaf growth and amount of herbivory would remain low.

### 4.4.2 Synchrony and elevation

In contrast to herbivory patterns in forest and tundra habitats, altitude had little influence on the synchrony between leaf development and the onset of herbivory. At both higher and lower elevations a lag of approximately one week was evident between the unfurling of leaf tissue and the onset of herbivory. Although synchrony may not be affected the proportion of leaf area removed by herbivores may increase slightly at higher elevations as tannin contents are low whilst tissue nitrogen concentration is high (Erelli et al., 1998). The lower tannin contents makes leaves more attractive to herbivores as tannins combine with plant proteins making them indigestible (Raven et al., 1999). The results of this study however showed that less leaf area was removed by herbivores at higher elevations. As the abundance of potential food plants at high elevation sites was reduced when compared to lower elevations (pers. obs.), herbivore numbers may also have been lower, something that could be tested in further studies.

### 4.4.3 Herbivory and plant life history

Deciduous plants experienced a higher degree of invertebrate herbivory across the forest-tundra ecotone than evergreen plants. Such a pattern would be expected as evergreen plants are pre-adapted to avoid herbivory as a result of xerophytic adaptations. The adaptations/defences include a thick cuticle, which provides an effective barrier to small mandibles, and glossy leaves that make it difficult for larvae to cling on to (Young, 1997). In contrast, deciduous plants do not invest so highly in defences, because the resources are required for repeated annual growth (Herms & Mattson, 1992) and are therefore more palatable to invertebrates.

Furthermore, similar to deciduous species, evergreen plants located at lower elevations had a higher proportion of herbivory. It is therefore likely that the invertebrate populations at higher elevations are limited by the altitude related winter minimum temperatures (Ruohomaki et al., 1997; Virtanen et al., 1998; Virtanen & Neuvonen, 1999). However, at lower elevations as competition will be higher between individuals it is likely that the invertebrates will consume any of the available plant species, particularly during outbreak years. The patterns of evergreen herbivory recorded in this study are likely to have incorporated herbivory that occurred in the previous year. Further studies could determine the actual rates of herbivory, for example by taking measurements only on newly unfurled leaves.

# Chapter 5 – Effects of warming on plant phenology and invertebrate herbivory

## 5.1 Introduction

Global circulation models (GCMs) predict that global mean temperatures will rise by 1.9 to 5.2 °C throughout the 21st century (Maxwell, 1992; Houghton et al., 1996). The predictions from the GCMs also suggest that the impacts of climate change will be greatest at high latitudes (Callaghan & Jonasson, 1995; Houghton et al., 1996). Understanding the implications of such predictions in relation to plant and animal communities has justifiably received much attention over recent years (Callaghan et al., 1992; Ayres, 1993; Whittaker, 2001; Bale et al., 2002).

An increase in mean temperature in Arctic regions is likely have many consequences for plant and animal communities (Hughes, 2000). The most prominent effect is likely to be the increased length of the growing season, because warmer temperatures will cause snow to melt earlier in the season (Callaghan & Jonasson, 1995). The combination of higher temperatures and a longer growing season is likely to result in increased growth rates for many plant species (Myneni et al., 1997; Cannell et al., 1998). Furthermore, an increase in mean global temperatures may result in a poleward shift of many species ranges as their northern range boundaries extend to higher latitudes (Smith, 1994; Parmesan et al., 1999). Similarly, the altitudinal range of species is likely to shift upwards, as climatic conditions ameliorate (Parmesan, 1996). Some of these changes in climatic conditions will potentially decouple phenological relationships between species, particularly when life cycles events are triggered by environmental cues, such as winter chilling requirements (Ellis et al., 1997; Pauli et al., 1998). Despite several studies, there is much debate as to whether climate change will in fact lead to phenological asynchrony between larval emergence and bud-burst (Watt & McFarlane, 2002; Dixon, 2003).

One such example is determining the impacts of increased temperatures on the interaction between invertebrate herbivores and their food plants, particularly in relation to outbreaks of *Epirrita autumnata* which can cause severe defoliation of the *Betula pubescens* ssp. *tortuosa* forest in Fennoscandia (Tenow, 1996). Many regions of the forest are currently subjected to high levels of invertebrate herbivory as bud-burst and egg hatching are closely synchronised (Klemola et al., 2003). However, Tenow & Nilssen (1990) found that certain areas of birch forest were protected from these outbreaks when winter temperatures fell below the supercooling point of *E. autumnata* eggs thereby killing them. If climate change were to increase the global mean temperatures, models have predicted that the areas which are currently protected from defoliation by *E. autumnata* larvae will be drastically reduced (Virtanen et al., 1998). In contrast, Virtanen & Neuvonen (1999) have suggested that the intensity of outbreaks in mountain birch forests will in fact decline because the frequency of larval parasitism will increase with warmer temperatures. Therefore, it is necessary to understand something about the ecology of the many components of the system before being able to predict with any degree of confidence the likely effects of climate change.

Understanding the effects of predicted climate change on the phenological synchrony between invertebrate herbivores and their host plants is also key to predicting whether outbreaks will become more or less frequent in the *B. pubescens* ssp. *tortuosa* forests of Fennoscandia. Researchers have used a variety of manipulations to simulate climate change and then study the effects of elevated temperatures on the functioning of Arctic ecosystems. Such techniques include infra-red heaters (Harte & Shaw, 1995), small climate controlled greenhouses (Tissue & Oechel, 1987) and plastic greenhouses (Debevec & MacLean, 1993). However, although these techniques may produce the desired changes in temperature there are problems associated with their use including: site damage; protection from the wind; altered light, humidity, photosynthesis, $CO_2$ concentrations and volume of incoming precipitation; trapping of snow; and creating a barrier to some invertebrate and vertebrate species (Debevec & MacLean, 1993;

Kennedy, 1995; Marion et al., 1997). Therefore, with many of these manipulations, experimentally increased temperature is also associated with other changes in the chambers relative to the controls, making it difficult to study the effects independently. In particular, most warming simulations cause snow melt to occur significantly earlier in the season, thereby extending the growing season length of experimental plots in comparison with control plots. The present study uses open-top chambers that do not significantly affect the date of snow melt, allowing these effects to be separated.

### 5.1.1 Hypotheses

#### 5.1.1.1 The effect of experimental warming on plant phenology and growth

It is generally accepted that a warmer climate in the Arctic will increase the length of the growing season by advancing the date of snow melt (Callaghan & Jonasson, 1995; Price & Waser, 1998; Serreze et al., 2000; Starr et al., 2000). Therefore, plant species that are closely synchronised with snow melt might respond by advancing their phenological development (Galen & Stanton, 1995). However, when snow melt is experimentally manipulated the hydrological conditions and temperature profiles are also affected, thereby making it difficult to establish which effect is responsible for influencing phenology. The present study investigates the effects of experimental warming on plant phenology without significantly manipulating date of snow melt.

In the current study, which examined several stages of leaf development, one would predict leaf development in OTCs to be accelerated relative to the control plots. In particular, even though the OTCs did not significantly alter the date of snow melt in relation to the control plots, we would predict that the warmer temperatures would advance the onset of bud-burst thereby shifting the entire period of leaf development to earlier in the season.

The effect of climate change on snow melt date and the phenology of plants has been well studied (for example Farnsworth et al., 1995; Price & Waser, 1998) however the implications of warming on leaf growth rates are less clear. Galen & Stanton (1993) for example, have shown that advancing the date of snow melt in habitually late melting areas of a snow bed led to a threefold increase in the percentage cover of snow buttercups (*Ranunculus adoneus*). However, a similar manipulation in early melting areas of the snow bed had little influence on the percentage cover. In contrast Galen & Stanton (1995) also found that species, such as *Sibbaldia procumbens,* whose leaf expansion is closely synchronised with snow melt, responded positively to early release from snow cover. This study tested the hypothesis that plants which are released from snow cover will commence their growth earlier and therefore have a larger leaf area as more time is available for growth. Furthermore, as this study has already shown that plants in forest habitats are able to develop faster than those in control plots in tundra habitats (see section 3.3.4.2 for details of the results) one would expect a similar pattern to occur in OTCs.

#### 5.1.1.2 The effect of experimental warming on invertebrate herbivory

If herbivores are able to respond to changes in plant phenology, we would expect the onset of invertebrate herbivory to be shifted forward, because leaf material is predicted to become available earlier in the growing season. Furthermore, if plants grow at an increased rate as a result of climate change, the leaves are likely to have a higher carbohydrate content causing the nutrient content to be diluted (Callaghan & Jonasson, 1995). Therefore, as invertebrate herbivores would need to consume a larger quantity of leaf material one would predict there to be higher levels of herbivory in OTCs than in control plots.

The present study has shown that the impacts of herbivory are greater in tundra habitats than in forest habitats (see section 4.3.1 for details of the results). As such patterns may simply be the result of the two habitats

having different abundances of potential host plants, we would also expect similar pattern to arise in OTCs.

If such predictions are correct, the impact of *E. autumnata* during an outbreak year would be higher than present, especially if the areas currently protected by cold air pockets are reduced (Tenow & Nilssen, 1990; Virtanen et al., 1998). However, it is vital that further studies are carried out investigate the likelihood of larval parasitism in order to determine the potential magnitude of destruction that would be caused by an outbreak of *E. autumnata* larvae (Virtanen & Neuvonen, 1999).

#### 5.1.1.3 The effect of experimental warming on synchrony

There is currently much debate as to whether climate change will affect the synchrony between insects and their host plants (Watt & McFarlane, 2002; Dixon, 2003). A study by Dewar & Watt (1992) concluded that the phenological synchrony that exists between the winter moth, *Operophtera brumata*, and *Picea sitchensis* would be disrupted in a warmer climate, while Buse & Good (1996) demonstrated the reverse with the same herbivore on *Quercus robur*. Therefore, shedding more light on how synchrony between insects and their host plants might be affected by warmer temperatures is vital in order to provide advice on managing future outbreaks. However, as the larvae that hatch closer to bud-burst will be fitter than those that hatch earlier or later it seems likely that selection will ultimately correct any break down in synchronisation (Dixon, 2003). Indeed, strong localised selection for hatching date was shown by Mopper & Simberloff (1995) and Van Dongen et al. (1997). However, uncertainty still exists as to whether selection can operate quickly enough to avert extinction of herbivore populations under climate change scenarios. Therefore, the current study tested the hypotheses that there will be a change in synchrony between invertebrate larvae and their host plants under experimental warming.

## 5.2 Methods

### 5.2.1 Experimental setup

A series of five open-top chambers (OTCs) was placed at each site across the forest-tundra ecotone in 1998. The OTCs, made from 5 mm thick UV-stabilised clear polycarbonate sheet, were hexagonal in shape and had an area of 1 $m^2$ at the base (see figure 2.4 on page 8). The OTCs were designed to magnify solar insolation and therefore increase the temperature within the OTCs in relation to the ambient temperature. Five control plots of the same area were marked out with twine and matched to the OTCs on the basis of aspect, slope and species composition, thereby allowing paired statistical analyses to be undertaken. Although previous work has been undertaken at these sites, including measurements of introduced sapling survival and point quadrat assessment of canopy composition and structure (see Huntley, 2002 for full details of experiments), disturbance levels have been kept to a minimum and no material has been removed.

### 5.2.2 Temperature measurements

To record the ambient temperatures experienced by invertebrates and their prostrate food plants, unshielded loggers were placed at ground level in a randomly selected OTC and control plot at each of the sites. The loggers were put in place before snow fall in autumn 2002 allowing temperatures to be recorded from 1 January 2003 to 14 June 2003. Due to memory limitations of the loggers, soil surface temperatures could not be recorded after 14 June 2003. Hourly soil surface temperatures were pooled to calculate daily mean, maximum and minimum temperatures. For analysis soil surface temperatures were divided into two groups: snow covered when soil surface temperatures were below 0°C; and snow free when soil surface temperatures rose and remained above 0°C.

### 5.2.3 Plant phenology

The timing and rate of leaf development were recorded across the forest-tundra ecotone to allow a comparison of phenology among the four study sites. Data were collected approximately every four days between the end of May 2003 and the beginning of July 2003 (see table 2.1 on page 11 for dates of each sampling session), resulting in nine sampling sessions. Twenty buds/leaves, from the appropriate species, were randomly selected in each plot using a random number table. The buds/leaves were categorised into 'bud', 'bud-burst', 'leaf visible' and 'leaf unfurled'. Leaf phenology measurements were taken at each session for a random sample of twenty bud-sites in each plot; see section 3.2.2 (page 20) for full details. As upper and lower forest plots were snow covered until late into the season any plots that were completely covered were noted and no further measurements were taken during the session. For analytical purposes leaves in plots which were covered by late lying snow were assumed to be at the same stage of development as they were when measurements were first taken.

### 5.2.4 Leaf area available and proportion removed by herbivores

To determine the leaf area available to invertebrate herbivores at each measurement session, the length of any fully unfurled leaves was measured using digital callipers. The measurements were taken from the tip of the petiole to the tip of the leaf (or estimated position of the tip if the distal portion had been removed by herbivory). When twenty or fewer leaves were present in a plot all leaves were measured, and when more than twenty were present, twenty were selected at random for measurement. The length measurements were then used to determine the predicted leaf area in the absence of herbivory using the regression equations given in section 2.4 (page 11). Mean leaf area was then multiplied by the number of unfurled leaves in each plot to determine the leaf area available to herbivores in each plot.

To determine the proportion of leaf area that had been removed by herbivores, a further twenty fully unfurled leaves were selected at random and examined for signs of herbivory. Where herbivory had occurred, diagrams of the damage on each leaf were recorded on pre-drawn templates of each plant species (see figure 4.1, on page 49, for examples of the templates). To determine the extent and development of herbivory the first twenty leaves showing evidence of herbivory were marked with a piece of cotton and a unique identification number. On subsequent visits the leaves were re-measured and new diagrams of herbivory were taken. The completed templates were digitised and calibrated to the size of each leaf before the actual area removed was calculated (see section 4.2.3 for further details, page 49).

### 5.2.5 Evergreen herbivory

The extent of invertebrate herbivory on *Vaccinium vitis-idaea* was recorded at all sites across the forest-tundra ecotone. In each plot, plants were randomly selected using a random number table and leaves were examined for any evidence of herbivory. The total number of leaves on each plant was recorded along with the number that had been attacked. A new plant was selected at random until 10 leaves with evidence of herbivory had been identified, a total of 500 leaves had been examined or all plants in the plot had been counted (see section 4.2.4 for further details, page 51). For statistical analyses, plants in plots covered by late melting snow were presumed to be protected from invertebrate herbivores and therefore the proportion of leaves with herbivory was assumed be the same as that for the first snow-free measurement period.

### 5.2.6 Statistical analysis

Leaf emergence was chosen as the comparative measure of phenology as the full cycle (i.e. from no leaves visible to all leaves visible) was encompassed for most sites during the measurement period. To calculate the mean date at which leaves began to emerge the proportion of leaves

which had reached the 'leaf visible' (see section 3.2.2 for definitions, page 20) stage of their development was determined for each measurement session and was multiplied by the Julian day. The sum of the weighted proportions was then divided by the sum of the actual proportion of leaves which were visible. The mean dates were then compared using Mann-Whitney tests. Using habitat, altitude and plot type as between-subject factors and leaf length at each session as the within-subject factor, a repeated measures ANOVA was used to investigate leaf growth patterns. To test whether synchrony between invertebrate larvae and their host plants was altered under experimental warming, the lag between leaf emergence and the onset of herbivory was calculated, by subtracting the date at which leaves emerged and the date at which herbivory commenced, and was then analysed using a two-way ANOVA.

## 5.3 Results

### 5.3.1 Soil surface temperatures

The soil surface temperature profiles recorded in OTCs were qualitatively similar to those in the control plots, although the quantitative effects varied across the forest-tundra ecotone (figure 5.1). The tundra sites were characterised by highly fluctuating temperatures throughout the entire measurement period while the forests sites showed a long period of stable soil surface temperatures when the sites were presumed to be covered by a thick isothermic snow pack (figure 5.1; see section 3.3.2 for a detailed description of the soil surface temperature profiles in relation to presumed snow cover). Despite the variability in soil surface temperatures recorded in OTCs and control plots, snow melt generally occurred at a similar time (table 5.1). The exception was in the upper forest site where snow melt in the OTC occurred five days earlier than in the control plot (figure 5.1 (b)).

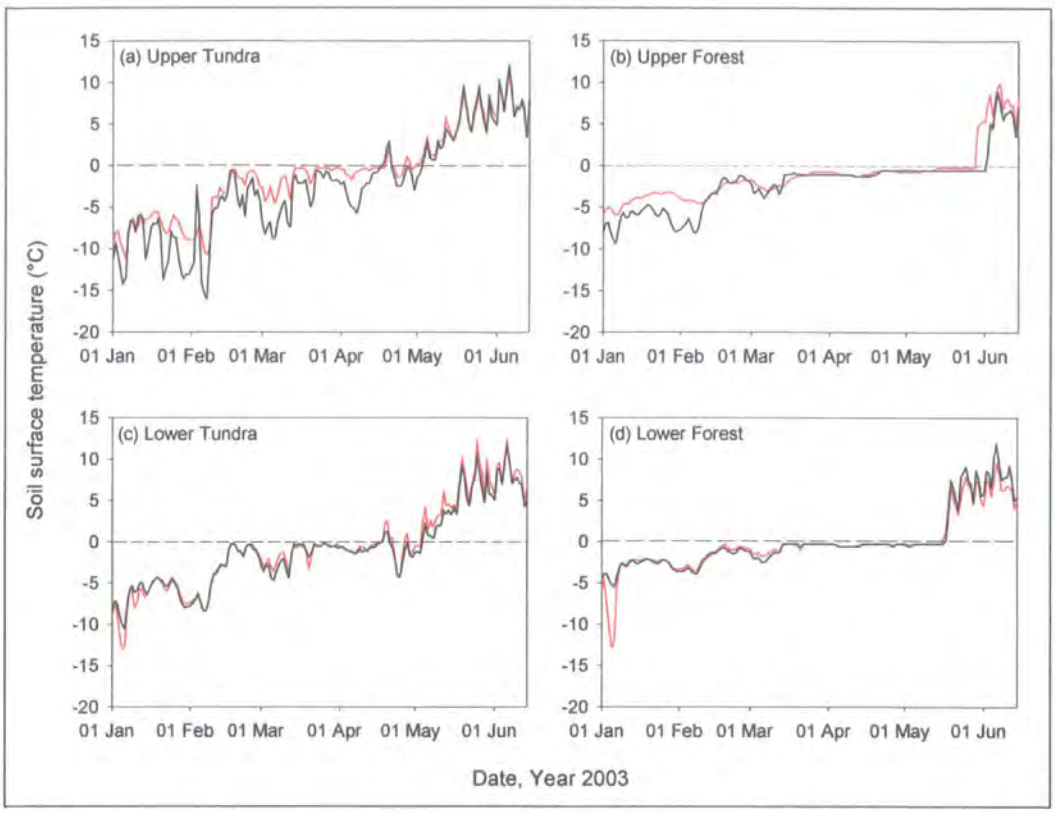


**Figure 5.1: Daily mean soil surface temperatures for (a) upper tundra, (b) upper forest, (c) lower tundra and (d) lower forest. Soil surface temperatures recorded in OTCs are shown with a red line while those from the control plots are shown with a black line.**

**Table 5.1: Date at which a randomly selected OTC and control plot was presumed to have become snow-free in the upper tundra, upper forest, lower tundra and lower forest sites. The snow-free period was defined by temperatures rising and remaining above 0°C (see section 3.3.2 for further details of determining periods of snow cover).**

| Altitude | Habitat | Control plot snow free date (2003) | OTC snow free date (2003) |
|---|---|---|---|
| Upper | Tundra | 3 May | 1 May |
| Upper | Forest | 2 June | 29 May |
| Lower | Tundra | 4 May | 4 May |
| Lower | Forest | 18 May | 16 May |

Soil surface temperatures measured in OTCs across the forest-tundra ecotone were generally greater than those measured in control plots (upper

tundra Wilcoxon test: $z = 3.535$, $n = 29$, $p < 0.001$; upper forest: $z = 3.223$, $n = 29$, $p = 0.001$; lower tundra: $z = 2.562$, $n = 29$, $p = 0.010$; figure 5.1). In these sites the average soil surface temperatures were 0.30 °C to 1.40 °C warmer in OTCs than in the control plots. The exception was in the lower forest where there was no difference in the soil surface temperatures recorded in the OTC and the control plot (Wilcoxon test $z = 0.597$, $n = 29$, $p = 0.550$).

During the period of presumed snow cover (see section 3.3.2 for details of estimating the period of snow cover) soil surface temperatures in the upper tundra, upper forest and lower tundra were warmer in the OTCs than in the control plots (Wilcoxon tests: all $p < 0.03$; table 5.2). In contrast, the soil surface temperatures in the lower forest site were warmer in the control plot than in the OTC (Wilcoxon test $z = 4.61$, $n = 135$, $p < 0.001$; table 5.2).

In the period after snow melt, soil surface temperatures in the upper forest and lower tundra plots were again warmer in the OTCs than in the control plots (Wilcoxon tests: all $p < 0.002$; table 5.2). On the upper tundra, however, there was no significant difference in the soil surface temperatures measured in the OTC and control plot (table 5.2). Similar to the snow covered period, the soil surface temperatures measured in the lower forest site were lower in the OTC than in the control plot (Wilcoxon test $z = -4.08$, $n = 28$, $p < 0.001$; table 5.2).

**Table 5.2: Minimum, maximum and mean soil surface temperatures (°C) with associated p values (Wilcoxon test) for the four study sites. Soil surface temperatures, collected between 1 January and 14 June 2003, were grouped into two phases: presumed snow cover (°C < 0) and snow free (°C > 0).**

| | Altitude | Habitat | Control plot | | | Open-top chamber | | | Mean warming (°C) | P value |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Min (°C) | Max (°C) | Mean (°C) | Min (°C) | Max (°C) | Mean (°C) | | |
| Snow Covered | Upper | Tundra | -16.03 | 2.88 | -5.27 | -11.15 | 2.20 | -3.40 | 1.87 | < 0.001 |
| | Upper | Forest | -9.41 | -0.60 | -2.80 | -6.00 | -0.26 | -2.15 | 0.65 | < 0.001 |
| | Lower | Tundra | -10.60 | 1.20 | -3.09 | -12.92 | 2.45 | -3.04 | 0.05 | 0.025 |
| | Lower | Forest | -5.53 | -0.29 | -1.47 | -12.83 | -0.25 | -1.54 | -0.07 | < 0.001 |
| Snow Free | Upper | Tundra | 0.04 | 12.09 | 5.58 | 0.96 | 11.09 | 5.70 | 0.12 | 0.196 |
| | Upper | Forest | 1.33 | 8.90 | 5.85 | 4.54 | 9.79 | 7.61 | 1.76 | 0.001 |
| | Lower | Tundra | 0.35 | 11.53 | 5.62 | 1.42 | 12.39 | 6.63 | 1.01 | < 0.001 |
| | Lower | Forest | 2.93 | 11.83 | 7.29 | 3.24 | 9.50 | 6.17 | -1.12 | < 0.001 |

### 5.3.2 Variation in the level of warming provided by OTCs

Open-top chambers generally increased the soil surface temperatures relative to control plots at higher elevations while having little effect at lower elevations (figure 5.2). In the upper tundra, for example, although the difference between the OTC and the control plot fluctuated extensively over the season, the soil surface temperatures were on average 1.40°C warmer in the former (figure 5.2 (a); see section 5.3.1 for details of soil surface temperatures). A similar pattern was observed at the upper forest site where, despite a long period from late-March to late-May when soil surface temperatures in the OTC were nearly the same as those in the control, the overall effect of the OTC was to increase the soil surface temperatures by 0.86°C (figure 5.2 (b); see section 5.3.1). The largest difference (around 1 June) in soil surface temperatures at the upper forest site reflects the

earlier melting of the snow cover in the OTC than in the control plot (figure 5.2; table 5.1).

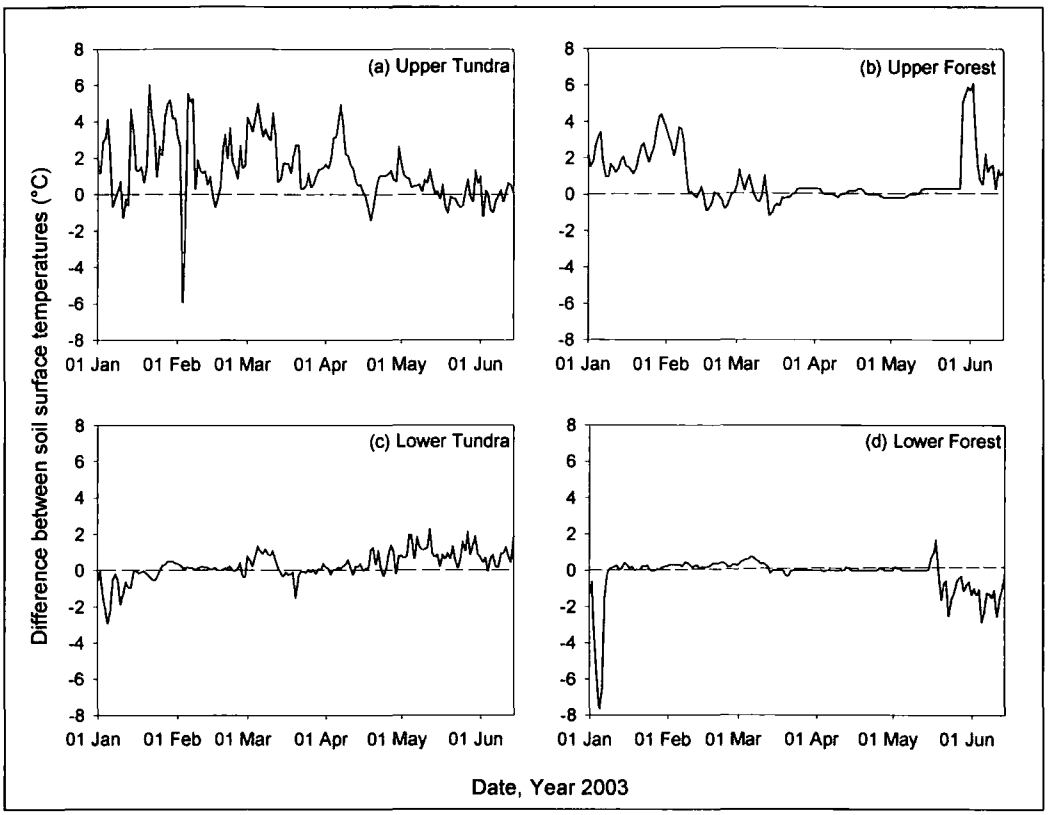


**Figure 5.2: Difference between daily soil surface temperatures measured in OTCs and control plots for (a) upper tundra, (b) upper forest, (c) lower tundra, and (d) lower forest (calculation: OTC daily soil surface temperatures – control daily soil surface temperatures). Positive values indicate that soil surface temperatures were warmer in OTCs than in control plots while negative values indicate that soil surface temperatures were warmer in control plots than in OTCs.**

In contrast, the differences between soil surface temperatures measured in the OTCs and control plots at lower elevations were much less pronounced (figure 5.2 (c) & (d)). In the lower tundra the soil surface temperatures were only 0.30°C greater in the OTC than in the control plot (figure 5.2 (c)). In the lower forest, however, the OTC had little effect on soil surface temperatures when the plots were snow covered (January to mid-May; see section 5.3.1 for details of soil surface temperatures). Once snow melt occurred, the soil surface temperatures were again lower in the OTC than in the control plot at this site; the mean difference between soil surface temperatures recorded in

the OTC and the control plot at the lower forest site was only 0.24°C (figure 5.2 (d); see section 5.3.1).

### 5.3.3 Plant phenological development

#### 5.3.3.1 Forest sites

The effect of growing in an OTC on the phenological development of *V. myrtillus* leaves in the forest plots varied markedly with elevation. At the upper forest site, even though bud-burst commenced at the same time in OTCs and control plots, leaf development was more rapid in OTCs, where soil surface temperatures were warmer than in the control plots (figure 5.3 (a) & (b)). Furthermore, the date at which leaves began to emerge in OTCs was ten days in advance of that in control plots (mean date for leaf visible in OTCs = 13 June, mean date for leaf visible in control plots = 24 June; $U_{104,50} = 207$, $n = 154$, $p < 0.001$; figure 5.3 (c)). *Vaccinium myrtillus* leaves in OTCs at the upper forest site had completed their development by 26 June, at least a week in advance of leaves in the control plots (figure 5.3 (d)). Therefore, elevated soil surface temperatures in OTCs appeared to increase the rate of leaf development in the upper forest site.

In contrast to the upper sites, the soil surface temperatures at the lower forest site were higher in the control plot than in the OTC. However, the onset of leaf development was earlier in OTCs than in control plots, with 60% of buds having burst in OTCs before measurements began, while only 10% had burst in control plots (figure 5.3 (e)). Despite this difference at the onset of leaf development, bud-burst was completed on the same date in both the OTCs and control plots (21 June; figure 5.3 (f)). Furthermore, there was little difference in the date at which leaves began to unfurl in OTCs and control plots (mean date for leaf visible in OTCs = 9 June, mean date for leaf visible in control plots = 10 June; $U_{236,177} = 18779$, $p = 0.07$; figure 5.3 (g)). Similarly, leaf development was completed at approximately the same time in both OTCs and control plots (figure 5.3 (h)). Therefore, in contrast to the

upper forest site, leaf development did not occur earlier in the control plots even though soil surface temperatures were higher than in the OTC.

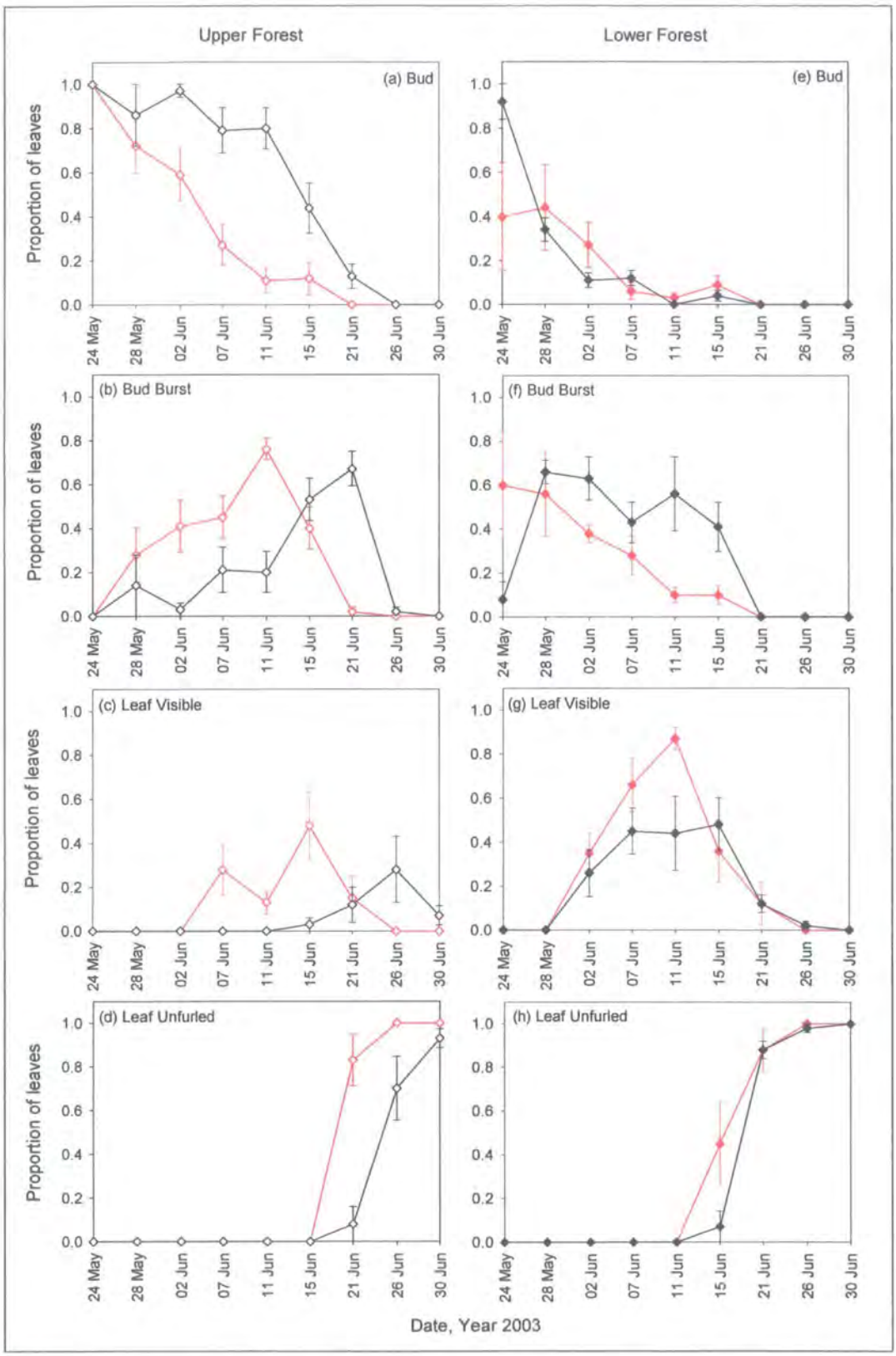


**Figure 5.3: Proportion of *V. myrtillus* leaves in bud, bud-burst, leaf visible and leaf unfurled phases of their development. Upper forest site is shown on the left with open diamonds ((a) to (d)) while the lower forest site is shown on the right with filled diamonds ((e) to (h)). At both elevations, OTCs are shown in red and control plots in black. Error bars are ± 1 SE.**

#### 5.3.3.2 Tundra sites

The effect of warming on the phenological development of *B. nana* in tundra sites varied markedly with elevation. At the upper tundra site, where soil surface temperatures were higher in the OTC than in the control plot, leaf development was more advanced in OTCs with 86% of buds having burst before measurements began, while only 76% had burst in control plots (figure 5.4 (a) & (b)). The mean date at which leaves in OTCs begun unfurling was also earlier than leaves in control plots (mean date for leaf visible in OTCs = 1 June, mean date for leaf visible in control plots = 5 June; $U_{200,255} = 19805.5$, $p < 0.001$; figure 5.4 (c)). The leaves in OTCs also completed their development earlier than those in control plots (figure 5.4 (d)), suggesting that the warmer soil surface temperatures recorded in the OTC influenced the entire development of *B. nana* leaves.

The pattern of *B. nana* leaf development at the lower tundra site was broadly similar to that at the upper site. Although soil surface temperatures were warmer in the OTC than in the control plot, leaf development commenced slightly earlier in the latter with 92% of buds having burst before measurements began, while only 84% had burst by this time in OTCs (figure 5.4 (e) & (f)). This difference was, however, soon reversed as the mean date at which leaves began to unfurl in OTCs at the lower tundra site was almost a week in advance of that in the control plots (mean date for leaf visible in OTCs = 31 May, mean date for leaf visible in control plots = 5 June; $U_{125,289} = 8134$, $p < 0.001$; figure 5.4 (g)). Finally, as leaf development in OTCs was completed almost one week earlier than in the control plots (figure 5.4 (h)), the results indicate that, like forest sites, OTCs were substantially accelerating leaf development in tundra sites.

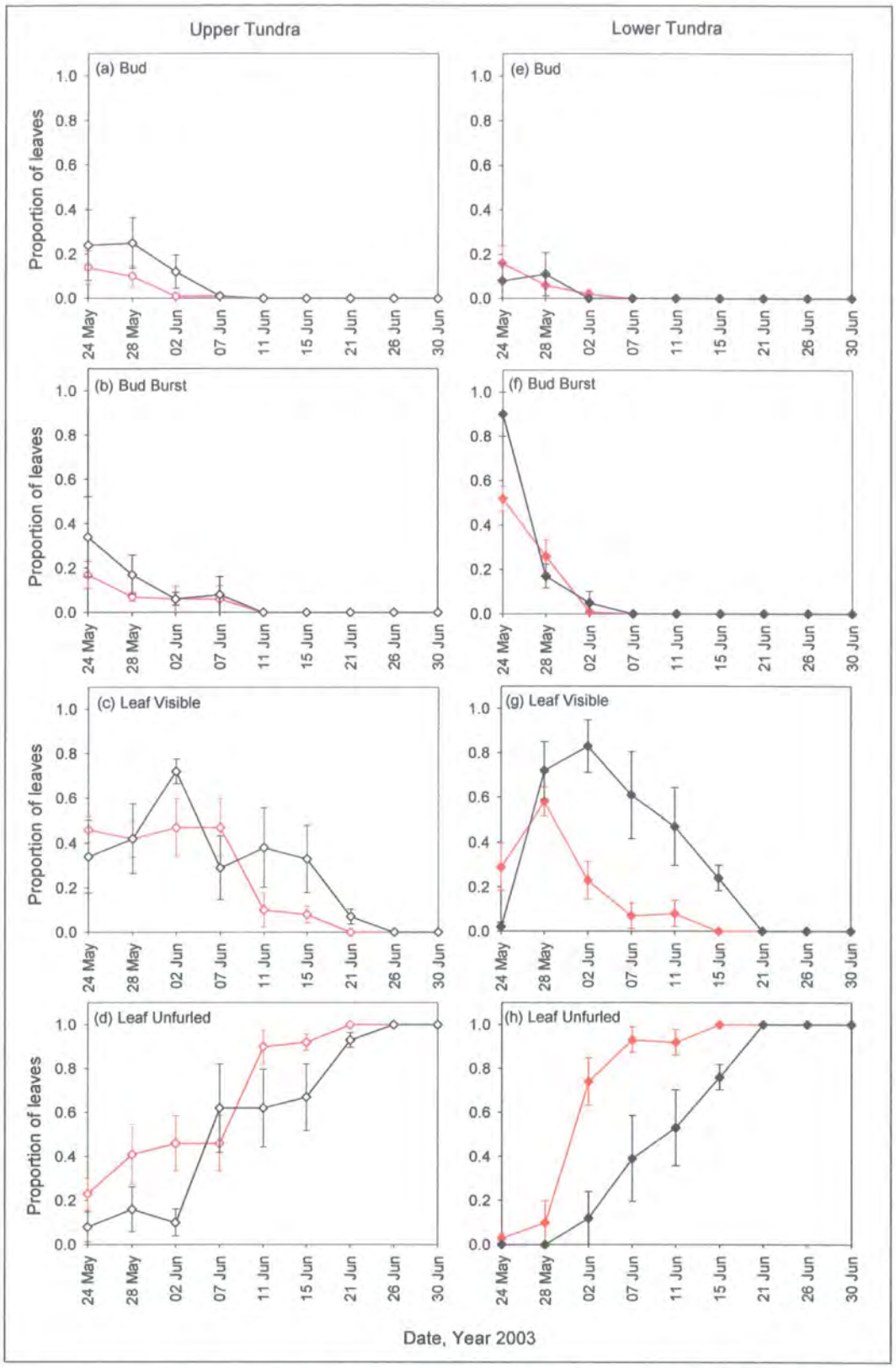


**Figure 5.4: Proportion of *B. nana* leaves in bud, bud-burst, leaf visible and leaf unfurled phases of their development. The upper tundra site is shown on the left with open diamonds ((a) to (d)) while the lower tundra site is shown on the right with filled diamonds ((e) to (h)). At both elevations OTCs are shown in red and control plots in black. Error bars are ± 1 SE.**

These results suggest that soil surface temperatures and leaf phenological development are closely linked and had both been manipulated by the experimental setup used here. In the three sites where the OTCs increased soil surface temperatures (upper tundra, upper forest and lower tundra) the development of leaves was advanced in comparison to the control plots. In contrast however, in the lower forest, where soil surface temperatures in the OTC were lower than in the control plot, there was no significant difference in the rate of leaf development.

#### 5.3.3.3 Does the warming effect depend on habitat?

In the forest sites, leaf development in OTCs and control plots followed a similar pattern (figure 5.5), although the mean date at which leaves began to unfurl in OTCs was significantly earlier than in control plots (mean date for leaf visible in OTCs = 10 June, mean date for leaf visible in controls = 13 June; $U_{171,115} = 7860.5$, $p = 0.003$; figure 5.5 (c)). Leaf development was completed at least four days earlier in OTCs than in control plots (figure 5.5 (d)).

The development of leaves commenced much earlier in the tundra sites than in the forest sites probably as a direct result of early release from snow cover (figure 5.5 (e); see section 3.3.2, page 23, for details of snow cover periods). The date at which leaves began to emerge in tundra sites was even earlier in OTCs than in control plots (mean date for leaf visible in OTCs = 31 March, mean date for leaf visible in controls = 4 June; $U_{144,274} = 11058.5$, $p < 0.001$; figure 5.5 (g)). Furthermore, over 90% of leaves in OTCs had completed their development by 7 June while only 50% had reached a similar stage in the control plots (figure 5.5 (h)). Therefore, leaf development in both forest and tundra habitats was advanced in OTCs in comparison with control plots.

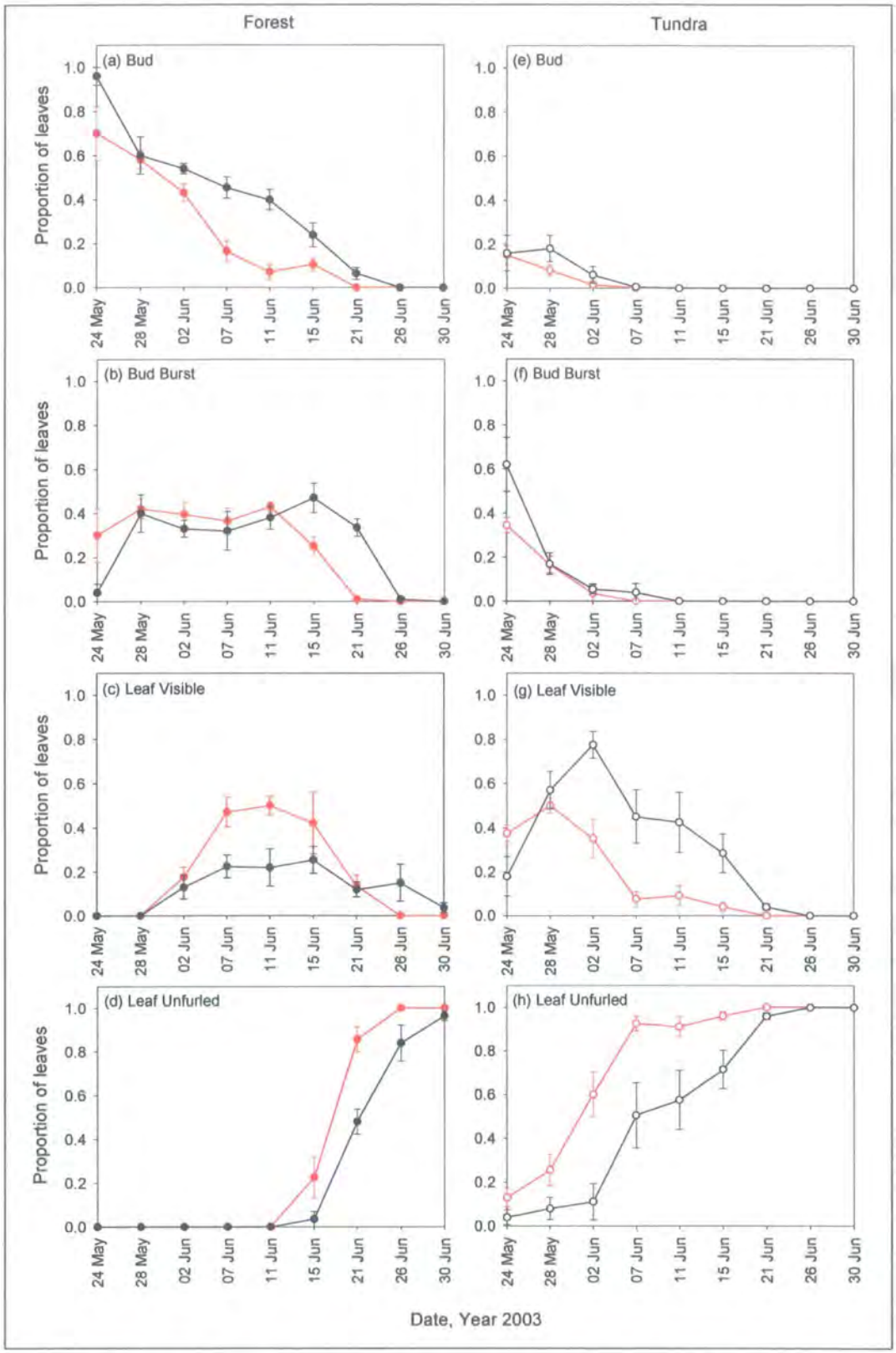


**Figure 5.5: Proportion of *V. myrtillus* leaves in forest sites ((a) to (d); filled circles) and *B. nana* leaves in tundra sites ((e) to (h); open circles) in bud, bud-burst, leaf visible and leaf unfurled phases of their development. For each habitat upper and lower sites were combined. OTCs are shown in red and control plots in black. Error bars are ± 1 SE.**

#### 5.3.3.4 Does the warming effect change with altitude?

In contrast to habitat, elevation did not strongly influence the difference in plant phenology between OTCs and control plots (see section 3.3.4.3, page 36). Firstly, at higher elevations, leaf development occurred at a similar rate in both OTCs and control plots (figure 5.6 (a) to (d)). Consequently, little difference was found between the dates at which leaves became visible in OTCs and control plots (mean date for leaf visible in OTCs = 5 June, mean date for leaf visible in control plots = 7 June; $U_{134,154} = 9090$, $p = 0.078$; figure 5.6 (c)). However, leaf development was completed slightly earlier in OTCs than in control plots; and the proportion of unfurled leaves was greater in OTCs than control plots in every session (figure 5.6 (d)).

Similarly plant phenology in control plots and OTCs at lower sites did not differ greatly (figure 5.6 (e) & (f)) and no difference was found between the date at which leaves began to unfurl in OTCs and control plots (mean date for leaf visible in OTCs = 5 June, mean date for leaf visible in control plots = 6 June; $U_{182,234} = 20019.5$, $p = 0.286$; figure 5.6 (g)). Furthermore, leaf development at the lower sites was completed at the same time in both OTCs and control plots (figure 5.6 (h)).

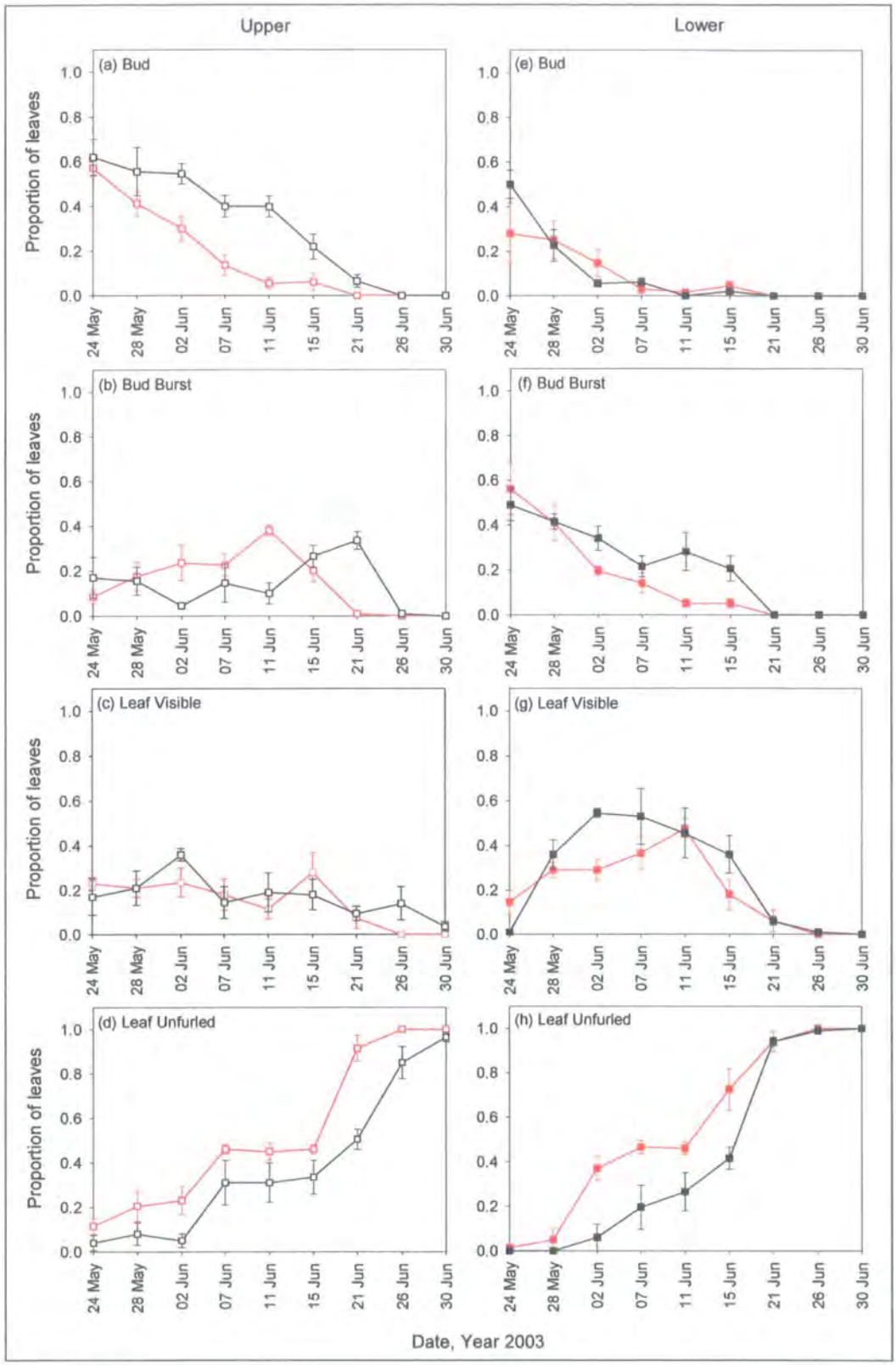


**Figure 5.6: Proportion of leaves at upper elevations ((a) to (d); open squares) and lower elevations ((e) to (h); filled squares) in bud, bud-burst, leaf visible and leaf unfurled phases of their development. For each elevation forest and tundra sites were combined. OTCs are shown in red and control plots in black. Error bars are ± 1 SE.**

### 5.3.4 Leaf growth

Although the qualitative patterns of leaf growth were similar in OTCs and control plots, leaves in OTCs at the upper tundra, upper forest and lower tundra sites appeared to grow at a faster rate and have a larger leaf area at any one time than those in control plots (figure 5.7 (a), (b) & (c)). However, in the lower forest site, there was little difference between the rate of leaf growth and leaf area in OTCs and control plots (figure 5.7 (d)).

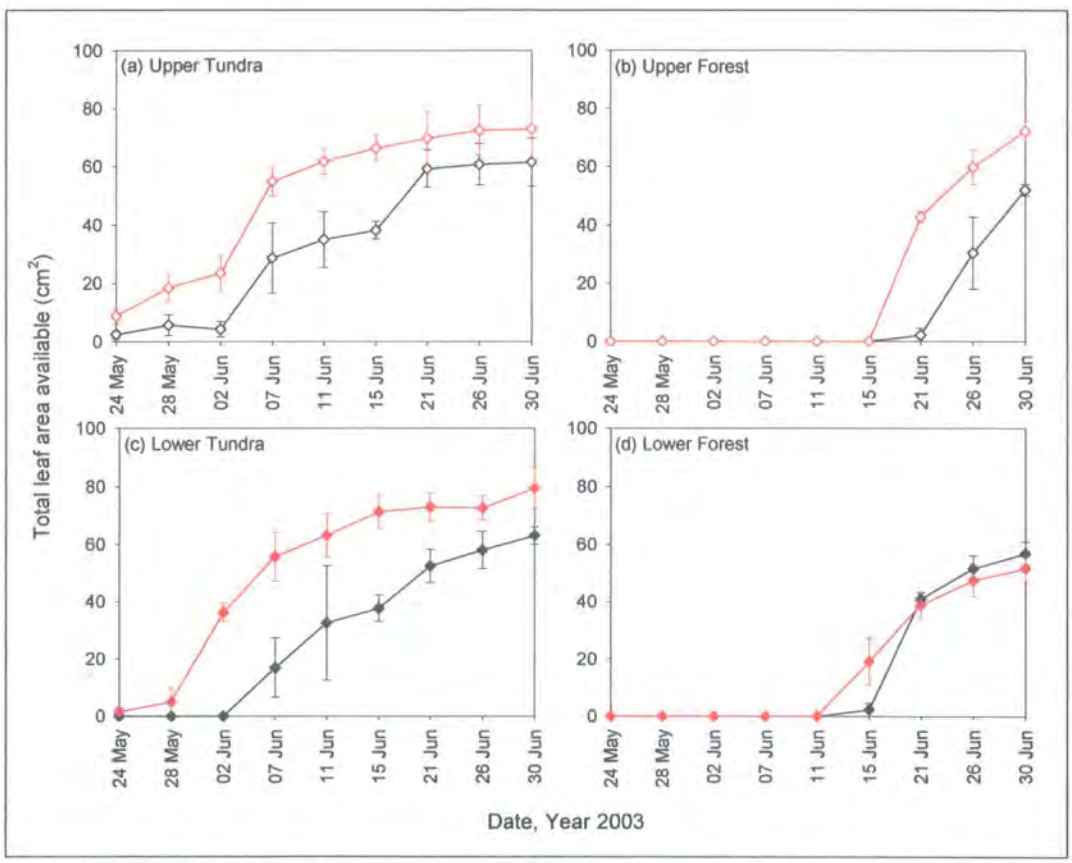


**Figure 5.7: Total leaf area available ($cm^2$) in five 1 $m^2$ plots in (a) upper tundra, (b) upper forest, (c) lower tundra, and (d) lower forest. Upper elevation sites are shown with open diamonds while lower elevations are shown with filled diamonds. OTCs are shown in red and control plots in black. Error bars are ± 1 SE.**

A repeated measures ANOVA was constructed to investigate the influence of habitat, elevation and warming on patterns of leaf growth. Habitat, altitude and plot type (OTC or control) were entered into the model as between-subject factors and leaf area at each session was the within-subject factor.

The results of the model are summarised in table 5.3. The model confirmed that, irrespective of the effects of OTCs, leaves in tundra sites had a larger area on any given session than those in forest sites ($F_{1,32} = 121.37$, $p < 0.001$; table 5.3), although there was no effect of elevation on leaf area ($F_{1,32} = 0.16$, $p = 0.692$; table 5.3; see section 3.3.4.3, page 36). Leaf area was greater in OTCs than in control plots ($F_{1,32} = 33.17$, $p < 0.001$; figure 5.7; table 5.3), and there was a strong interaction between chamber and habitat, with leaf area being increased in OTCs to a greater extent in tundra than forest habitats ($F_{1,32} = 9.79$, $p = 0.004$; table 5.3). The effect of OTCs did not vary with significantly with elevation ($F_{1,32} = 0.37$, $p = 0.546$; table 5.3).

**Table 5.3: Results of the repeated measures ANOVA used to investigate the influence of habitat, elevation and warming on leaf area available.**

| | Type III sum of squares | F | P |
|---|---|---|---|
| Habitat | 2177.13 | 121.37 | < 0.001 |
| Chamber | 595.00 | 33.17 | < 0.001 |
| Altitude | 2.87 | 0.16 | 0.692 |
| OTC * Habitat | 175.66 | 9.79 | 0.004 |
| Habitat * Altitude | 20.46 | 1.14 | 0.294 |
| OTC * Altitude | 6.69 | 0.37 | 0.546 |
| OTC * Habitat * Altitude | 57.43 | 3.20 | 0.083 |

### 5.3.5 Leaf area removed by herbivores

The amount of leaf tissue removed by herbivores varied extensively across the forest-tundra ecotone, with herbivory commencing earlier and remaining at a higher level in tundra sites than in forest sites (figure 5.8).

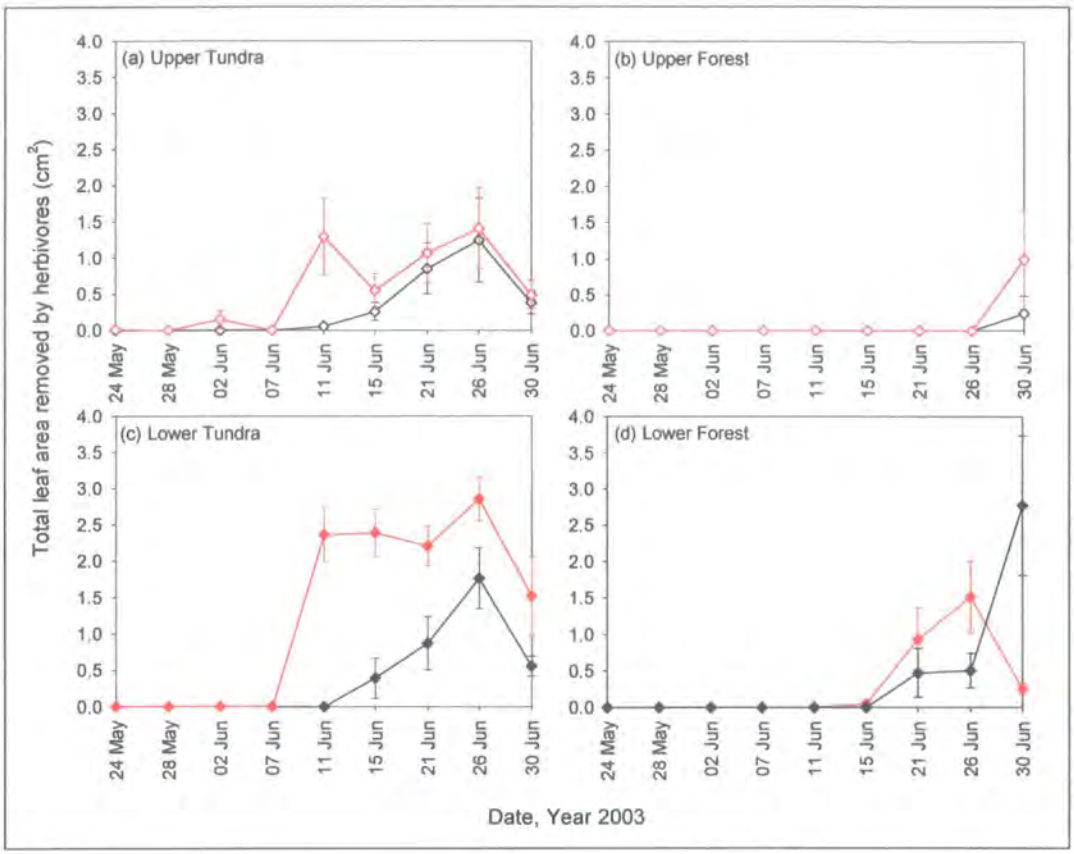


**Figure 5.8: Total leaf area removed by herbivores ($cm^2$) in (a) upper tundra, (b) upper forest, (c) lower tundra, and (d) lower forest. The total sample was twenty leaves in each plot per sampling session, and values are combined for the five OTCs and control plots in each site. OTCs are shown in red and control plots in black. Error bars are ± 1 SE.**

A repeated measures ANOVA was again used to investigate the relative influence of habitat, elevation and warming on the leaf area removed by herbivores. Habitat, altitude and plot type (OTC or control) were entered into the model as between-subject factors and leaf area removed at each session was the within-subject factor. The results of the model, which are summarised in table 5.4, showed that the amount of leaf area removed by herbivores was greater in OTCs than in control plots ($F_{1,32} = 8.05$, $p = 0.008$). After controlling for the effects of OTCs, plants in forest habitats suffered lower levels of herbivory than plants in tundra habitats ($F_{1,32} = 15.62$, $p < 0.001$; table 5.4). The amount of leaf tissue removed was increased in OTCs to a greater extent in tundra than in forest habitats ($F_{1,32} = 7.19$, $p = 0.012$; table 5.4). Elevation also had a significant effect on leaf area

removed, irrespective of the effects of OTCs, as plants at the lower sites suffered higher levels of herbivory than those at the upper sites ($F_{1,32} = 8.28$, $p = 0.007$; table 5.4). The effect of OTCs did not vary significantly with elevation ($F_{1,32} = 0.90$, $p = 0.351$; table 5.4).

**Table 5.4: Results of the repeated measures ANOVA used to investigate the influence of habitat, elevation and warming on leaf area removed by herbivores.**

| | Type III sum of squares | F | P |
|---|---|---|---|
| Chamber | 0.308 | 67.54 | 0.008 |
| Habitat | 0.60 | 15.62 | < 0.001 |
| Altitude | 0.32 | 8.28 | 0.007 |
| OTC * Habitat | 0.28 | 7.19 | 0.012 |
| OTC * Altitude | 0.03 | 0.90 | 0.351 |
| Habitat * Altitude | 0.0001 | 0.005 | 0.946 |
| OTC * Habitat * Altitude | 0.09 | 2.34 | 0.136 |

### 5.3.6 Proportion of available leaf area removed by herbivores

As well as available leaf area and total leaf area removed, the proportion of leaf area removed by herbivores was also greater in OTCs than in control plots ($F_{1,32} = 9.11$, $p = 0.005$). However, regardless of the effects of OTCs, a greater proportion of leaf area was removed by herbivores at lower elevations (mean % removed in lower plots = 1.01%; mean % removed in upper plots = 0.40%; $F_{1,32} = 17.67$, $p < 0.001$) and in tundra habitats (mean % removed in tundra plots = 1.06%; mean % removed in forest plots = 0.42%; $F_{1,32} = 21.74$, $p < 0.001$). Finally, there was a strong interaction between chamber and habitat, with the proportion of leaf area removed being increased in OTCs to a greater extent in tundra than forest habitats ($F_{1,32} = 6.19$, $p = 0.018$).

### 5.3.7 Patterns of synchrony between plants and herbivores

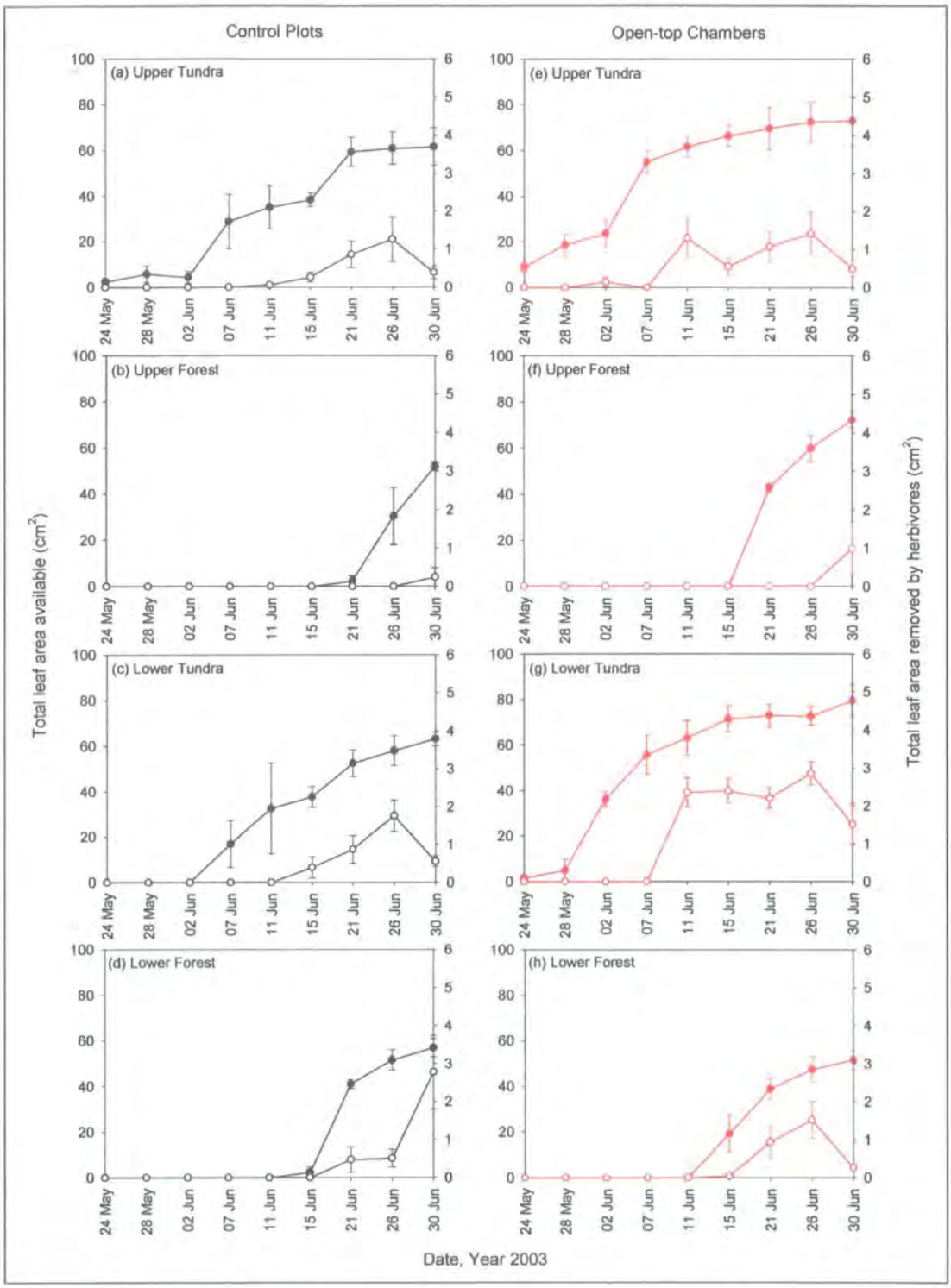


**Figure 5.9: Total leaf area available in control plots (black filled circles) and OTCs (red filled circles), and the total leaf area removed by herbivores in control plots (black open circles) and OTCs (red open circles) in the four study sites. Error bars are ± 1 SE.**

As discussed earlier, leaf material became available earlier in OTCs than in control plots at all sites across the forest-tundra ecotone (figure 5.9; see section 5.3.4 for details of leaf growth). A two-way ANOVA was used to investigate whether the effects of site and experimental warming (fixed factors) influenced the lag between leaf emergence and the onset of herbivory (dependent variable). The results of the model demonstrated that the lag between the emergence of leaves and the onset of herbivory was longer in tundra sites than in forest sites ($F_{3,31} = 3.76$, $p = 0.025$), irrespective of the effects of warming treatments. Since leaves at the upper tundra site had begun to emerge before measurements started (see figure 5.9 (a) & (e)), it is likely that the time lapse before the onset of herbivory is a rather conservative estimate. Furthermore, there was no difference in the lag between emergence of leaves and the onset of herbivory ($F_{1,31} = 0.32$, $p = 0.58$) in the OTCs relative to the control plots.

### 5.3.8 Herbivory of evergreen plants

As might be expected for an evergreen plant, the proportion of *V. vitis-idaea* leaves that showed evidence of invertebrate herbivory remained at a relatively low level throughout the measurement period. Plants in tundra sites appeared to be subjected to higher levels of herbivory than those in forest sites particularly in the OTCs (figure 5.10).

A repeated measures ANOVA was used to determine whether habitat, altitude and warming affected the proportion of leaves that had been attacked by herbivores. Habitat, altitude and plot type (OTC or control) were entered into the model as between-subject factors and the proportion of leaves with herbivory at each session was the within-subject factor. The results of the model showed that there was significantly more herbivory on evergreen plants at lower elevations than at higher elevations, irrespective of the effects of OTCs ($F_{1,32} = 35.19$, $p < 0.001$). Furthermore, excluding the effects of OTCs, there was no influence of habitat on the proportion of evergreen leaves that had been attacked ($F_{1,32} = 2.51$, $p < 0.123$).

Finally, as in the deciduous plants, the levels of herbivory on evergreen plants were found to be greater in OTCs than in control plots ($F_{1,32} = 9.20$, $p = 0.005$). However, the effect of OTCs varied with habitat and altitude. As figure 5.10 clearly demonstrates, the impact of OTCs on invertebrate herbivory were greater in tundra sites than in forest sites ($F_{1,32} = 20.62$, $p < 0.001$) and in lower elevations than in upper elevations ($F_{1,32} = 4.18$, $p = 0.049$). Therefore, invertebrate herbivory on both evergreen and deciduous plants was higher at the lower tundra site in both OTCs and control plots.

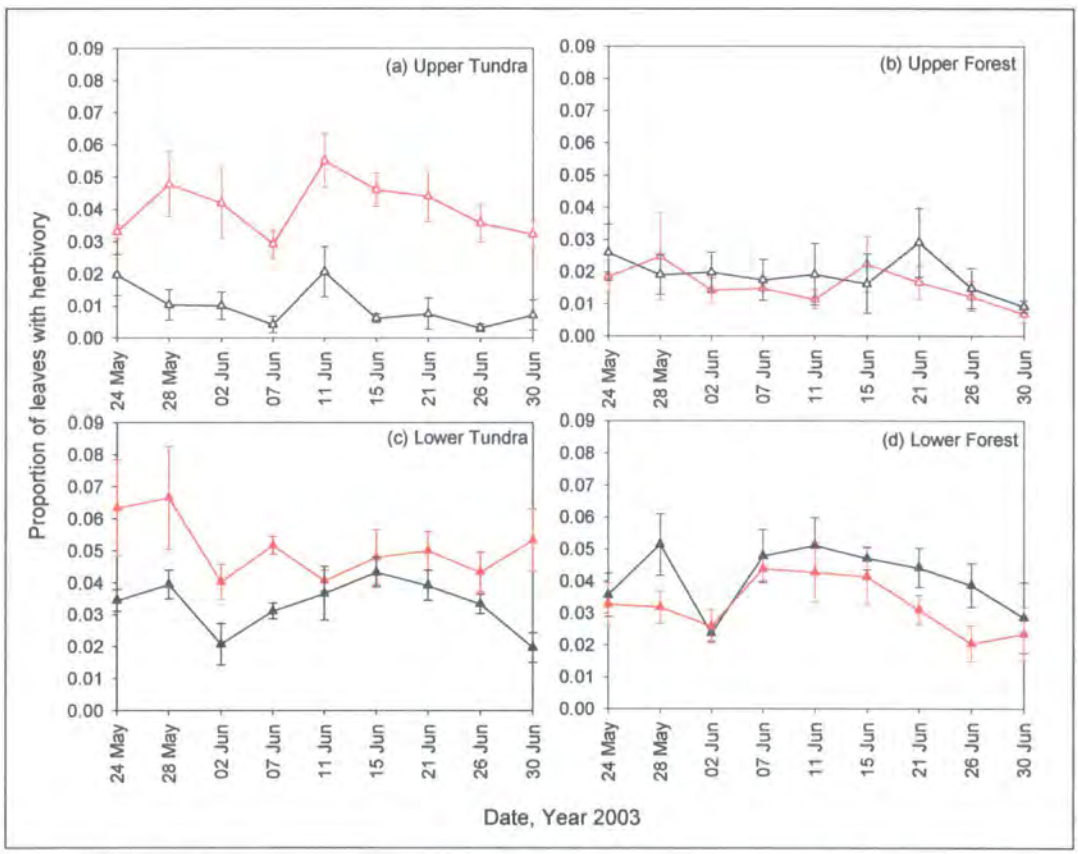


**Figure 5.10: Proportion of evergreen leaves with herbivory in (a) upper tundra, (b) upper forest, (c) lower tundra, and (d) lower forest. Upper elevation sites are shown with open triangles while lower elevations are shown with filled triangles. OTCs are shown in red and control plots in black. Error bars are ± 1 SE.**

## 5.4 Discussion

### 5.4.1 Soil surface temperatures

Open-top chambers were generally found to increase the soil surface temperatures relative to those recorded in the control plots. The amount of warming was broadly similar to that predicted by GCMs. These factors, taken together, point to substantial amelioration of climatic conditions by the OTCs. The magnitude of warming was largest in OTCs located at the upper sites. As the upper sites were the most exposed, it is possible that the OTCs were acting as a wind break thereby increasing the ambient temperatures relative to the control plots (Marion et al., 1997). However, in the lower forest site there was no difference in the soil surface temperatures which were recorded in the OTC and in the control plot. Since the lower forest site was located in a lee area within the relatively dense birch forest it is possible likely that the OTCs trapped the cold air. Furthermore, the OTCs may have been shaded from direct sunlight, by canopy cover, thereby limiting any potential warming through insolation.

Although the soil surface temperatures were warmer in the OTCs than in the control plots, there was generally little difference in the date at which snow melt occurred. The exception was in the upper forest site where snow melt occurred five days earlier in the OTC than in the control plot. As the dense snow pack in forest sites did not melt until ambient temperatures were well above freezing (see table 5.1, page 71, for snow melt dates and section 3.3.1, page 22, for air temperature records) it is likely that the OTCs were able to trap some of this heat and thereby speed up the melting process. Therefore, unlike many studies that manipulated snow packs (for example: Kudo, 1991; Galen & Stanton, 1993; Galen & Stanton, 1995; Starr et al., 2000), one can conclude that plant phenology and invertebrate herbivory patterns were not affected by hydrological differences in the present study.

### 5.4.2 Plant phenology

Similar to previous studies, plant phenology was advanced in the OTCs that had warmer soil surface temperatures than the control plots (Farnsworth et al., 1995; Starr et al., 2000; Post et al., 2001). However, since bud-burst had commenced at all sites, except the upper forest, before measurements began it is not possible to determine whether the onset of leaf development was advanced by warmer temperatures. Even so, the development of leaf tissue was advanced in these OTCs relative to the control plots suggesting that plant phenology responded positively to increased temperatures. At the upper forest site, however, the fact that bud-burst occurred at the same time in the OTCs and control plots suggests that plants in the OTCs were able to speed up the rate of their development in comparison with those in the control plots. As there was no difference in the timing of plant phenology at the lower forest site it appears that leaf development is controlled by thermal conditions rather than the days since snow melt. Therefore, in common with many alpine plants, those used in this study have developmental, morphological and physiological adaptations that promote rapid initiation of growth under favourable conditions (Bliss, 1985).

As a result of the thick isothermic snow pack that persisted until late in the season in both forest sites, the development of leaf tissues was delayed relative to the tundra sites. There was however, no difference in the timing of plant phenology at different elevations. This study suggests that *V. myrtillus* and *B. nana* are able to respond positively to the increased temperatures by increasing the rate of their development thereby taking advantage of a longer growing season.

### 5.4.3. Leaf growth

Both *V. myrtillus* and *B. nana* leaves had a larger leaf area in OTCs than in control plots. This result contrasts with the findings of several other studies, which demonstrated that leaf area was not affected by experimental warming (for example: Starr et al., 2000; Totland & Alatalo, 2002). However, as these

studies also manipulated snow pack it is possible that leaf growth may have been limited by soil moisture (Price & Waser, 2000). Because there was little difference in the date of snow melt in OTCs and control plots in the present study it is possible to conclude that warmer temperatures alone stimulate increased growth in active plants. This study only examined the early part of the growth period and further studies should determine whether this effect extends to later in the season.

The results of the present study also indicated that leaf area was larger in tundra habitats than in forest habitats in both OTCs and control plots. This pattern is likely to have arisen because tundra sites were released from snow cover much earlier in the season thereby providing the plants with a longer growing season in comparison to those in forest sites. Although leaves in tundra habitats had a larger leaf area than those in forest sites, plants in the latter habitat had a faster rate of development than those in the former habitat. Finally, the leaf area of *V. myrtillus* and *B. nana* plants was not influenced by elevation.

### 5.4.4 Invertebrate herbivory

Invertebrate herbivores had a larger impact when more leaf tissue was available, i.e. in OTCs and in both OTCs and control plots located in tundra habitats. Higher levels of invertebrate herbivory are believed to be a common side-effect of increased plant growth because nutrient levels are diluted by the extra carbohydrates (Callaghan & Jonasson, 1995). In areas where herbivory levels were increased one can either assume that larger numbers of herbivores were present or that the densities of herbivores were similar to the control plots but the individuals were consuming a larger proportion of available material. Larvae could continuously consume leaf tissue if not constrained by their digestive enzymes, which work more efficiently at higher temperatures (Young, 1997). Therefore, it is possible that density of invertebrate herbivores was similar in both OTCs and control plots but the elevated temperatures of OTCs increased digestion rates thereby allowing herbivores to consume a larger amount of the available leaf

tissue. However, as levels of herbivory were higher in tundra habitats than in forest habitats, irrespective of the effects of OTCs, it is possible that larvae were simply responding to the reduced nutrient content of more rapidly grown leaf tissue. Further studies need to be undertaken to distinguish between these hypotheses and determine whether population densities of invertebrates differed across the forest-tundra ecotone.

### 5.4.5 Phenological synchrony

Even though leaf material became available earlier in OTCs than in control plots the length of time that elapsed before the onset of herbivory was similar in both experimental treatments. In other words, *E. autumnata* larvae were able to maintain phenological synchrony with their host plants despite elevated temperatures. These results support the findings of Buse & Good (1996) who concluded that a general increase in temperature would not affect the degree of synchrony between egg hatch of winter moth (*Operophtera brumata*) and bud-burst of *Quercus robur*. However, since the experimental plots in the present study have been in place for four years it is possible that there may have initially been asynchrony between egg hatch and bud-burst which has since corrected itself as a result of strong selection pressure (i.e. high mortality rates for those that hatch too early or too late).

The findings of the present study, however, contrast those of Dewar & Watt (1992) who demonstrated that the synchrony between the winter moth, *Operophtera brumata,* and *Picea sitchensis* may be disrupted with climate change. In strong contrast to relationships with deciduous hosts, the winter moth has been shown to survive if they hatch before bud-burst on *P. sitchensis* (Watt & McFarlane, 1991). As it is therefore evident that phenology does not always limit larval abundance it is perhaps most important to determine how herbivores track their hosts (Dixon, 2003) in specific cases before general rules can be established. For example, although the gall-forming aphid, *Hormaphis hamamelidis* (Fitch), hatches before bud-burst of their food plant, *Hamamelis virginiana* L., to be sure of obtaining immature foliage (Rehill & Schultz, 2002), Arctic herbivores that

follow a similar strategy would be subject to extreme cold at the beginning of the season.

### 5.4.6 Evergreen herbivory

The proportion of evergreen leaves attacked by invertebrate herbivores was found to be higher in OTCs than in control plots. Furthermore, *V. vitis-idaea* plants located in tundra sites were subject to higher levels of herbivory than those in forest sites in both OTCs and control plots. Such patterns may simply be the result of the tundra sites becoming snow free before the forest sites thereby allowing more time for herbivory. Alternatively, the increased rates of herbivory in OTCs may again be related to the higher temperatures which would potentially influence digestion rates (Young, 1997). However, as these results incorporate herbivory for previous years any further studies should examine the levels of herbivory on newly unfurled leaves.

# Chapter 6 – General Discussion

This study has demonstrated that the phenology of Arctic plants and their associated herbivores was strongly influenced by warming. Warmer temperatures not only increased the rate of leaf development, but also resulted in larger leaf area in comparison to those in control plots, suggesting an increase in production. Furthermore, a larger proportion of available leaf material was removed by herbivores in OTCs than in control plots, but there was no difference in the synchrony between bud-burst and egg hatching as a result of the experimental treatment. These results suggest that the severity of defoliation events across the Fennoscandia mountain chain may increase under predicted climate change scenarios, because the invertebrate herbivores were not only able to adjust the timing of their eclosion in relation to the availability of leaf tissues but were also able to consume a larger proportion of the available material.

## 6.1 Plant phenology and growth in a changing climate

Given that the leaf biomass of both *Vaccinium myrtillus* and *Betula nana* increased under simulated warming, the growth of these species appears to be limited by environmental conditions. The most likely of these is temperature. This result makes intuitive sense, because low biomass production in Arctic regions is primarily limited by the short growing season rather than slow growth (Sonesson & Callaghan, 1991; Grace et al., 2002). Although several studies have demonstrated that increased temperatures lead to an increase in biomass production in vascular plants (for example Chapin & Shaver, 1985; Juntilla & Nilsen, 1993; Chapin et al., 1995; Myneni et al., 1997), this is not the case for all plant species. For example, working on *Polygonum bistorta* L., Starr et al. (2000) found no change in leaf size in plants subjected to soil warming. Similarly, Suzuki & Kudo (1997) demonstrated that the leaf area of several alpine plant species, including *Vaccinium uliginosum* and *Arctous alpinus* (L.) Niedenzu, was not affected by an increase in temperature generated using OTCs. Clearly, climatic warming

may not affect all species equally (Press et al., 1998). Species whose growth is limited by internal constraints may be at a competitive disadvantage as the climate warms, particularly where the rate of selection is limited because of clonal proliferation (Wookey et al., 1993). There is a need to examine more species over the kind of timescale used in the present study, to identify any taxonomic or functional correlates of responses to climate change. Only then will we be able to predict the consequences of climate change for entire communities.

The date of snow melt is generally considered to be the main environmental cue that initiates growth in many Arctic plant species (Sorensen, 1941; Billings & Bliss, 1959; Galen & Stanton, 1993; Galen & Stanton, 1995; Walker et al., 1995). The increases in temperature associated with climate change will undoubtedly affect the timing of snow-melt in the spring and thereby alter the start date of the growing season (Maxwell, 1992; Van Wijk et al., 2003). Price & Waser (1998) suggested that such changes in the timing of snow melt would cause an immediate phenological shift in plant communities. Such patterns have indeed been recorded for various plant species (for example Callaghan et al., 1992; Suzuki & Kudo, 1997; Hughes, 2000; Starr et al., 2000; Post et al., 2001; Walther et al., 2002). However, few studies have examined the effects of temperature on Arctic plant phenology without significantly altering the timing of snow melt. The results of the present study have demonstrated that the rate of leaf development is accelerated by increased temperature, even when there is no difference in the timing of snow melt.

Since measurements were only taken at the beginning of the growing season in the present study, it is not possible to determine whether the timing of senescence was also altered. Starr et al (2000) showed that although *Polygonum bistorta* responded to increased temperature by becoming active earlier, the date of senescence was also advanced, and the length of the growing season for plants in experimental plots was similar to that for plants in the control plots. However, small differences in temperature early in the season can have profound effects on subsequent plant growth (Grace et al.,

2002), and the relationship between plants and herbivores depends on the timing of events occurring early in the season, when leaf tissue is palatable. This study provides clear evidence that elevated temperatures early in the growing season increase the rate of plant development independent of the effects of changes in the timing of snow melt.

## 6.2 Invertebrate herbivory and synchrony in a changing climate

The proportion of available leaf material that was removed by invertebrate herbivores increased in warmed plots. Invertebrate herbivores are known to be limited by the rate at which food can be digested rather than the rate at which it can be consumed (Young, 1997). Because the efficiency of digestive enzymes increases with temperature, the raised levels of herbivory demonstrated in this study are not unexpected. However, as increased temperatures have been shown to stimulate plant growth it is likely that the nitrogen content of the leaves will be diluted (Laine & Henttonen, 1987; Michelsen et al., 1996; Hartley et al., 1999), thereby forcing the herbivores to consume more leaf material in order to obtain the required nutrients. As the effects of warming on leaf quality are thought to be species-specific (Richardson et al., 2002) it is essential to determine how leaf nutrient quality is affected by warming on a case-by-case basis. In the present study, because it was not possible to measure leaf nitrogen content, no conclusions could be drawn as to whether climatic warming allows invertebrate herbivores to voluntarily increase their rate of tissue consumption and thereby speed up their developmental rate, or whether the diluted nutrient content forces them to consume a larger quantity of material.

Ultimately, however, invertebrate herbivores are still constrained by the need to consume young foliage that has a low tannin concentration (Feeny, 1970) and will therefore face selection pressure to synchronise their emergence with the onset of bud-burst (Ayres & MacLean, 1987). Despite several recent studies and reviews, there is still much confusion as to whether a warmer climate will lead to phenological asynchrony between organisms at different trophic levels (Watt & McFarlane, 1991; Dewar & Watt, 1992; Buse & Good,

1996; Harrington et al., 1999; Hughes, 2000; Visser & Holleman, 2001; Watt & McFarlane, 2002; Dixon, 2003). During the initial climatically induced adjustments to the timing of bud-burst, any invertebrates that are well synchronised to the new conditions will have a higher fitness than those that emerge either too early or too late (Dixon, 1976; Harrington et al., 1999; Visser & Holleman, 2001; Dixon, 2003). Therefore, as more of the surviving individuals will have inherited responses that enable them to track the phenology of their host plants, proportionally more of future generations will emerge in synchrony with bud-burst (Dixon, 2003). However, as many of the past studies only examined the possible implications of increased temperatures in relation to synchrony over a short time period, it is likely that only the immediate responses were being recorded, possibly overestimated the long term effects of climate change. Longer term studies indicated that there may be a long time lag between the initiation of treatment and ecosystem response (Chapin et al., 1995), so it is essential that studies with a longer time frame are undertaken to investigate how climate change may affect trophic interactions. This study has shown that phenological synchrony between Arctic plants, specifically, *V. myrtillus* and *B. nana*, and their associated herbivore was not disrupted after temperatures had been raised experimentally for a period of five years.

Although several studies have investigated the potential impacts of climate change across different trophic levels, most of them have elevated the temperatures by 2 – 5 °C (for example Dewar & Watt, 1992; Buse & Good, 1996; Richardson et al., 2002). As such increases in temperature are only predicted to come about after several decades of climatic warming it is clear that any conclusions drawn from these studies may overestimate the severity of the effects of climate change. The level of warming achieved in this study (average of 0.44 °C across all sites) is still higher than that predicted by GCMs in a five year period (Houghton et al., 1996), but it provides a more realistic indication of how plants and their associated herbivores may respond to climate change. In light of this it is perhaps of greater importance to undertake heritability studies to determine the maximum rate at which

populations can respond to climatically induced directional selection at different trophic levels.

## 6.3 Implications for the forest of northern Fennoscandia

As the birch forests of northern Fennoscandia have been subjected to severe defoliation events approximately every 9 to 10 years (Tenow & Bylund, 2000) there is undoubtedly a need to identify any possible consequences of climatic warming. The present study has demonstrated that although increased temperatures may advance the onset of bud-burst and stimulate leaf growth, the invertebrate herbivores are expected to remain in synchrony with bud-burst and to consume a higher proportion of the available leaf material. Therefore, because more of the available leaf material will be removed by herbivores under expected scenarios of climate change, defoliation events may be expected to increase in severity in the future.

Recent studies have, however, identified several complicating factors that may also affect the amount of herbivory. For example, Virtanen & Neuvonen (1999) demonstrated that larval parasitism may increase in warmer climates and thereby decrease the intensity of any potential outbreaks. Similarly, if the rate of leaf growth was increased in a warmer climate the length of time during which high quality immature foliage is available to herbivores will also be reduced (Pollack, 1990; Kuokkanen et al., 2003), potentially decreasing the impact of herbivores. In contrast, several patches of forest are currently protected from invertebrate outbreaks as a result of cold air accumulations that kill overwintering eggs. If winter warming takes place according to the predictions made by GCMs, the areas protected by cold air accumulations may be significantly reduced (Virtanen et al., 1998). It is therefore evident that a wide range of factors is likely to mediate the impacts of climate change across different trophic levels and therefore conclusions from individual studies should be interpreted with caution.

## 6.4 What next?

The thrust of this work has been to reveal how the synchrony between Arctic plants and their associated herbivores may be affected by climatic warming. In order to gain a clearer picture the next stage of this research must concentrate on the rate at which plants and their associated herbivores are able to adapt to changes in their environment.

As it is generally believed that increased temperatures will lead to a decrease in leaf quality as a result of stimulated growth (Laine & Henttonen, 1987; Michelsen et al., 1996; Hartley et al., 1999), measurements should be taken to confirm whether this is indeed the case for *Vaccinium myrtillus* and *Betula nana*. This would be of great importance to determine whether invertebrates are forced to consume a larger proportion of the leaf tissue in order to obtain the required nutrients or whether they are voluntarily increasing their rate of consumption.

Furthermore, as the OTCs were not closed environments, mark-recapture experiments should be undertaken on the invertebrates to determine whether the individuals causing the damage are in fact being subjected to warming. Finally, although the soil surface temperatures provide us with an indication of the temperatures experienced by many of the plant and invertebrate species, the ambient air temperatures experienced within the experimental plots may be much higher. Therefore, since the herbivores are frequently elevated from the soil surface it would be worthwhile supplementing this with records of the ambient air temperatures.

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